Title: The effects of a 12 day intensified training period on the exercise- induced salivary hormone and mucosal immune responses

Name: Michael Harrison

THE EFFECTS OF A 12 DAY INTENSIFIED TRAINING PERIOD ON THE EXERCISE- INDUCED SALIVARY HORMONE AND MUCOSAL IMMUNE RESPONSES

By

Michael Harrison BSc (Hons)

A thesis submitted to the University of Bedfordshire, in fulfilment of the requirements for the degree of Masters of Science by Research at the Institute for Sport and Physical Activity Research (ISPAR)

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Author's Declaration

I, Michael Harrison declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

The effects of a 12 day intensified training period on the exercise-induced salivary hormone and mucosal immune responses

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Abstract

The present study investigated the exercise-induced salivary hormonal (cortisol and testosterone) and mucosal immunological (salivary secretory immunoglobulin A (SIgA)) responses to a 30 min high-intensity running bout ($RPE_{treadmill}$) before and after (~1 day and 6 days after) a 12 day intensified training period. Eight active males completed the study. Unstimulated saliva samples were collected pre-exercise, post-exercise and 30 min post-exercise and assessed for salivary cortisol, testosterone and SIgA. 10 km TT performance decreased by 3% from pre- to post-training (ES = 0.24). The exercise-induced salivary testosterone response significantly elevated by 36% pre-training and decreased by 42% post-training (ES = 0.43). Salivary cortisol and SIgA responses were unaffected by the exercise and training, although a small ES between pre- and post-training were observed (0.32-0.49). Four participants completed a post-recovery trial and salivary cortisol, testosterone, SIgA responses were unaffected by the exercise and training, although a small to large ES between pre-training, post-training and post-recovery were observed (0.4-1.24). Individual results are presented for four participants and two were identified as showing signs of overreaching. This study demonstrates that the $RPE_{treadmill}$ bout induces robust elevations in salivary testosterone and can potentially highlight any alterations in this hormone following a 12 day intensified training period.

Key words: salivary testosterone, salivary cortisol, salivary secretory immunoglobulin A, overreaching, intensified training

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List of Abbreviations

%	Percentage
~	Approximately
<	Less than
>	Greater than
±	Plus-minus
°C	Degrees Celsius
55/80	30 min continuous cycle consisting of 1 min at 55% $\dot{W}_{max}$ and 4 min at 80% $\dot{W}_{max}$
60/90	30 min continuous cycle consisting of 1 min blocks at 60% $\dot{W}_{max}$ and 90% $\dot{W}_{max}$
ACTH	Adrenocorticotrophic hormone
ANOVA	Analysis of variance
beats·min^{-1}	Beats per minute
CBG	Cortisol-binding globulin
cm	Centimetre
ELISA	Enzyme linked immunosorbent assay
ES	Effect size
FOR	Functional overreaching
GnRH	Gonadotrophin-releasing hormone
h	Hours
HPA	Hypothalamic-pituitary-adrenal
HPG	Hypothalamic-pituitary-gonadal

HR	Heart rate
HR_{max}	Maximum heart rate
IgA	Immunoglobulin A
kg	Kilogram
km	Kilometre
$km \cdot h^{-1}$	Kilometres an hour
LH	Luteinizing hormone
min	Minute
mL	Millilitre
n	Number
NFOR	Non-functional overreaching
$nmol \cdot L^{-1}$	Nanopoles per litre
OTS	Overtraining syndrome
pIgR	Polymeric immunoglobulin receptor
$pmol \cdot L^{-1}$	Picomoles per litre
RESTQ-76 Sport	Recovery stress questionnaire for athletes questionnaire
RPE	Ratings of perceived exertion
$RPE_{treadmill}$	30 min running bout alternating between blocks of 1 min at 11 RPE and 4 min at 15 RPE
s	Second
SCN	Suprachiasmatic nucleus
SHBG	Sex hormone-binding globulin
SIgA	Salivary secretory immunoglobulin A

TMB	Tetramethylbenzidine
TRIMP	Training impulse
TT	Time trial
URS	Upper respiratory symptoms
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}_E$	Minute ventilation
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max}$	Maximum oxygen consumption
$\dot{W}_{max}$	Maximum power output

CHAPTER 1 Introduction

Optimising physical performance is a fundamental aim for athletes to achieve during a competitive season (Mujika, 2010). To elicit this, carefully planned training programmes are designed in order to achieve peak performance that coincide with competition days to maximise the potential for success (Borresen and Lambert, 2009). Therefore, training periodisation is commonly used to structure and plan training and includes phases of insufficient training, competitive training, active rest and tapering (Plisk and Stone, 2003). The two most important components of a successful training programme are the training stress (intensity, frequency and volume) and recovery. Increasing the training stress and incorporating this with sufficient recovery induces positive physiological adaptations such as muscle hypertrophy to occur and therefore may help to improve the athlete's physical performance (Armstrong and VanHeest, 2002). However, an imbalance between the training stress and the recovery increases the likelihood of injury and possibly lead to the overtraining syndrome (OTS) resulting in chronic maladaptation's and performance decrements (which may never fully recover to baseline) (Meeusen *et al.*, 2013).

Overreaching and the OTS are viewed on a continuum from acute fatigue (short-term recovery) to chronic fatigue (long-term recovery) as a result of intensified training overload (Figure 1). Short-term overreaching is defined as functional overreaching (FOR) which occurs in the acute stages of fatigue. When the training intensity is increased, the recovery time becomes longer in duration resulting in long-term overreaching defined as non-functional overreaching (NFOR) (Meeusen *et al.*, 2013). Additional intensified training without inadequate recovery prolongs the recovery process further and can move into an extreme state of fatigue defined as the OTS (Meeusen *et al.*, 2013). It has been suggested that signs of overreaching can occur within a period as short as 7 days of intensified training with limited recovery (Halson *et al.*, 2002). Symptoms of overreaching and the OTS also include prolonged fatigue, psychological and hormonal disturbances (Hooper, Mackinnon and Hanrahan, 1997), loss of motivation (Meeusen *et al.*, 2013), loss of appetite, unexplained weight loss and sleep disturbances (Armstrong and VanHeest, 2002). These symptoms of overreaching are common in elite athletes with studies showing prevalence rates of up to 64% and career rates of up to 33% in non-elite athletes (Birrer *et al.*, 2013). The prevalence rate of the OTS is less common due to retrospective diagnosis however the severity of OTS is still a problem for athletes as many who have developed OTS are at a heightened risk of relapsing

(Meeusen *et al.*, 2013). Therefore, the importance of preventing overreaching (leading to the OTS) occurring is crucial for athletes as prolonged periods of these symptoms can interfere with optimal preparation for major competitions (MacKinnon, 2000).

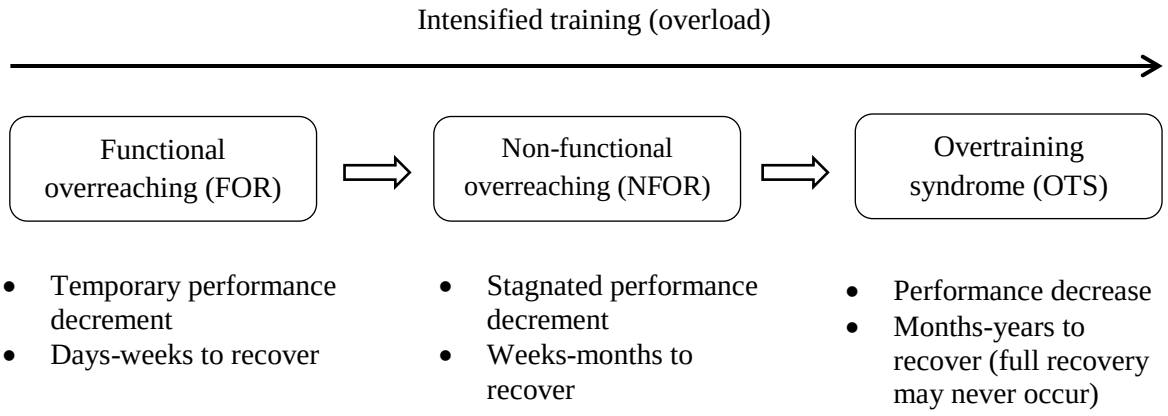

Figure 1 Schematic representation of overreaching (FOR and NFOR) and the overtraining syndrome (OTS) continuum (adapted from Meeusen *et al.* (2013)).

Currently there is no biological marker available to diagnose and aid in the prevention of overreaching and therefore establishing a reliable detection method is both useful and required (Meeusen *et al.*, 2013). The most recent position statement from the European College of Sport Science and American College of Sports Medicine on OTS has outlined a number of key ingredients of a reliable biological marker (Meeusen *et al.*, 2013). Firstly, a reliable biological marker should be sensitive to the training load without being affected by other factors such as diet and chronobiological rhythms. Changes in the marker should occur before the onset of OTS as well as being able to distinguish between acute and chronic responses to exercise. Inexpensive, non-invasive and ease of measurement with quick availability of results are also key ingredients to consider. Finally, obtaining the marker should be from rest or submaximal exercise of relatively short-duration so it does not disrupt an athlete's training programme (Meeusen *et al.*, 2013). Establishing a reliable biomarker of overreaching would therefore help to predict/monitor athletes prospectively for the incidence of the OTS. Many markers have been suggested and these include hormonal alterations (Duclos, 2008), performance measures (Meeusen *et al.*, 2004) and immunological alterations (Halson and Jeukendrup, 2004; Kreher, 2016). However, to date, none of these

markers have been shown to provide sufficient sensitivity and reliability to be used as a diagnostic test of overreaching.

Hormonal alterations of cortisol and testosterone have been highlighted as potential indicators of overreaching and the OTS (Duclos, 2008). These hormones have previously been examined in relation to overreaching and the OTS as they have been shown to be indicative of stress and recovery in response to exercise leading to disruptions in the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axis (Kreher, 2016). Early research extensively investigated the resting concentrations of testosterone/cortisol ratio concluding that the use of both hormones as definitive markers of the OTS are equivocal with no clear and consistent alteration in these markers to highlight a state of overreaching (Meeusen *et al.*, 2013). Due to the disparity in the literature regarding the usefulness of using resting measures of cortisol and testosterone to highlight overreaching/OTS, the examination of the exercise-induced hormonal alterations of cortisol and testosterone responses during an overreached state has recently been investigated (Meeusen *et al.*, 2004, 2010; Hough *et al.*, 2011, 2013; Hough, Robertson and Gleeson, 2015). These studies have shown encouraging results and may provide a useful measure to highlight a state of overreaching. However, only a limited amount of research is available that addresses this research area.

Other markers of overreaching and the OTS have been investigated, specifically immunological responses due to alterations in immunological function of athletes when training excessively (Kreher, 2016). Periods of intensified training (leading to overreaching and the OTS) creates an "open window" for illnesses such as upper respiratory symptoms (URS) (Halson and Jeukendrup, 2004; Meeusen *et al.*, 2013). In accordance with the latest Position Statement on Immune Function and Exercise, URS is now the accepted term to use in exercise studies rather than upper respiratory tract infections (URTI) (Walsh *et al.*, 2011). Although URTI are the most common reason for the appearance in athletes, recent evidence has suggested that not all URTI that occur in response to training are due to an "open window" of impaired immunity. Therefore, there is evidence to suggest other hypotheses for non-infectious causes of URS (i.e. airway inflammation, allergy and autoimmune disorders) (Helenius, Lumme and Haahtela, 2005; Cox *et al.*, 2008). Furthermore, URTI are verified by physician confirmation and this has come under scrutiny and been found to be less ideal (Cox *et al.*, 2008).

The “open window” hypothesis proposes that between 3 and 72 h after intensive exercise, athletes are susceptible to illness (Pedersen and Ullum, 1994). Many researchers have expanded on this hypothesis suggesting that athletes who engage in excessive amounts of intensive exercise without a sufficient recovery period show a cumulative effect of the “open window”. In principle, this chronically alters the immune function of athletes and could therefore explain the immunosuppression in overreaching athletes and athletes with OTS (Mackinnon, 1999; Smith, 2003). This phenomenon has been modelled in the form of a J-shaped curve which suggests that moderate levels of exercise reduces the risk of URS while chronic levels of exercise increases the risk of URS (Nieman, 1994b, 1994a). Subsequently, research has focused its attention on the mucosal immune system as it has been reported that 95% of all reported URS are originated from the mucosal surfaces (Bosch *et al.*, 2002). In particular, salivary secretory immunoglobulin A (SIgA) is used as the primary marker of the mucosal immune system as the majority of immunoglobulins are expressed in mucosal fluids (MacKinnon, 2000; Owen *et al.*, 2016). SIgA is the only immunological marker that consistently shows an association with URS (Gleeson, 2000; Bishop and Gleeson, 2009; Shephard, 2010).

CHAPTER 2 Literature Review

This chapter will describe the notion of overreaching and the OTS and specifically review the hormonal (cortisol and testosterone) and immunological (SIgA) markers of overreaching. This chapter will then review the current literature on the cortisol and testosterone responses to acute exercise and periods of intensified training. Moreover, a review of the literature on the SIgA responses to acute exercise and periods of intensified training will follow.

2.1 Overreaching and the Overtraining Syndrome

For athletes to develop and improve their performance, a successful training programme must include overload and recovery (Borresen and Lambert, 2009). Therefore, an imbalance between the two needs to be avoided to ensure that an abnormal training response does not occur (Meeusen *et al.*, 2013). Athletes will commonly increase the intensity and/or volume of the training in order to enhance performance (Mujika, 2010). Consequently, acute feelings of fatigue may be experienced as a result of a single intense training session or intense training period. Athletes will progressively overload the body by increasing the intensity and/or volume of training, which is likely to cause the athlete to underperform for a limited period of time (days to weeks). But with sufficient recovery, the athlete will return or experience a *super-compensatory* effect where performance is enhanced beyond baseline levels. This phenomenon is known as functional overreaching (FOR) (Meeusen *et al.*, 2013). This is what coaches would strive to achieve to improve an athletes performance (Halson, 2014). However, if an athlete continues to complete an intensified training load without completing appropriate recovery then they move into a state of overreaching known as non-functional overreaching (NFOR) (Meeusen *et al.*, 2013). Recovery from this state may take weeks to months to complete (Meeusen *et al.*, 2013). In extreme cases where inadequate recovery is incorporated, there is potential for OTS to develop and this could take months or years for full recovery from this syndrome. It is also possible that full recovery never occurs (Meeusen *et al.*, 2010). However, much of the literature reviewed shows that NFOR and OTS is very difficult to distinguish between as athletes will often show similar signs and symptoms of performance decrement and hormonal and psychological disturbances (Meeusen *et al.*, 2004). More importantly, the main difference is the duration of time it takes for full recovery to occur from underperformance (i.e. FOR, days to weeks, NFOR, weeks to

months and OTS, months to years) and this may not occur at all if an athlete is suffering from OTS (Meeusen *et al.*, 2010). The term "prolonged maladaptation" of biological, neurochemical and hormonal regulation mechanisms may be useful in diagnosing athletes with OTS (Meeusen *et al.*, 2013). Also, the subtle difference between NFOR and OTS is made more complicated by the fact that clinical features are individualised and are nonspecific, anecdotal and numerous (Meeusen *et al.*, 2013).

2.2 Markers of Overreaching – Hormone Responses

As no definitive marker has been established to diagnose athletes with FOR, NFOR and OTS, the need for establishing a biological marker of overreaching specifically is necessary. This will help reduce the incidences of OTS if the measurement is understood and acted upon by coaches and athletes. The literature suggests that cortisol (catabolic in nature) and testosterone (anabolic in nature) can be indicative of a state of stress and recovery respectively (Duclos, 2008). The foundation of the research in this area suggested that resting plasma testosterone/cortisol ratios were considered as an indicator of overreaching (Meeusen *et al.*, 2013). It was thought that the testosterone/cortisol ratio decreases in response to the intensity and duration of training and therefore being indicative of an overreached (FOR or NFOR) state. However, recent literature has shown this not to be a true indication of overreaching/OTS as evidence shows that the testosterone/cortisol ratio only indicates the actual physiological strain of exercise (Duclos, 2008). Urhausen, Gabriel and Kindermann (1998) investigated the hormonal responses in 17 male endurance athletes prior to and during a state of short-term overtraining over a period of ~19 months. They found that resting testosterone/cortisol ratios did not significantly change between normally trained (i.e. healthy) and overtrained athletes (defined as decrease in performance and early fatigue) (cortisol 254 ± 19 vs 264 ± 28 $nmol \cdot L^{-1}$; testosterone 17.2 ± 1.5 vs 18.4 ± 2.2 $pmol \cdot L^{-1}$). However, a moderate effect size (ES) for testosterone was observed which does highlight that a difference between normally trained and overtrained athletes did occur but were not identified as significant due to a lack of power of the study. Similarly, Mackinnon *et al.*, (1997) using 24 elite swimmers observed no significant difference in the resting plasma testosterone/cortisol ratio between overreached and well-trained athletes following a 4 week intensified training period. Supplementary studies have also observed no changes in the resting testosterone/cortisol ratios which further highlights that this marker may be incapable

of identifying athletes in an overreached state (Flynn *et al.*, 1994; Hooper *et al.*, 1995). In these studies, participants were classified as overreached if they displayed decreases in performance and persistent feelings of fatigue. Therefore, the use of the testosterone/cortisol ratio as a marker to evaluate overreaching in these subjects was found to be inconsistent.

It is clear from previous studies that the resting testosterone/cortisol ratio as a marker of overreaching and OTS is not reliable and also highlights that this ratio cannot distinguish between NFOR, FOR and OTS. Furthermore, it is evident in endurance athletes who have a normal 24 h cortisol secretion during rest (Duclos, Gouarne and Bonnemaison, 2003), morning plasma cortisol concentrations and 24 h urinary cortisol excretion is similar to those of similar aged sedentary subjects (Duclos *et al.*, 1997). Furthermore, they also maintain a seasonal rhythmicity of cortisol excretion similar to sedentary men (Meeusen *et al.*, 2006). Many of the studies investigating hormonal responses highlights the variation of methods used suggesting that diagnosing athletes with overreaching or OTS is difficult due to pre-analytical factors such as sample timing, food intake, gender, age and also other hormones which are under a feedback control (Meeusen *et al.*, 2013). Therefore, a consensus in the literature suggests that overreaching and the OTS must be viewed on a continuum with a disturbance, an adaptation, and maladaptation of the HPA and HPG axes (Urhausen, Gabriel and Kindermann, 1998; Meeusen *et al.*, 2004; Kreher, 2016).

In recent years, some attention has been given to examining the exercise-induced hormone responses as this may provide a better understanding of neuroendocrine imbalances that occur during an overreached state (Meeusen *et al.*, 2004, 2010). Meeusen *et al.* (2004) developed a test protocol involving two consecutive maximal incremental exercise tests separated by 4 h. Using seven well-trained cyclists, a state of overreaching was induced during a training camp (10 day training camp including an increase in training volume of 58% from a normal training volume). Meeusen and colleagues found that cycling performance (time to volitional exhaustion) had decreased by 6% between first and second exercise tests in the overreached group and there was also a blunted cortisol and adrenocorticotrophic hormone (ACTH) response following the 10 day training period compared with athletes who were in a normal trained state. This study suggests that endocrine responses are altered to short-duration and high-intensity exercise while in an overreached state. A follow up to the previous study was conducted investigating whether a distinction between NFOR and the OTS could be made (Meeusen *et al.*, 2010). It was expected that the cortisol response is less pronounced in a FOR stage and significantly blunted in a NFOR stage whereas individuals with the OTS would be

expected to have complete cortisol suppression (Meeusen *et al.*, 2004). Using the same two-bout protocol, they found that cortisol responses were good markers for OTS but were much less sensitive to distinguish between NFOR and OTS. The two-bout cycling protocol has shown positive results and could be used as a tool to diagnose overreaching and OTS, however, the two exercise tests separated by 4 h could be influenced by diurnal variation of the hormones and also bouts are very long in duration (i.e. 6 h to complete). Therefore, a single short-duration, high-intensity exercise test would be more useful due to the simple practicality of completing a shorter test (i.e. 30 min) compared to completing two 40 min tests separated by 4 h.

A continuous 30 min high-intensity cycling bout, compromising of alternating blocks of 1 min at 55% maximum work rate ($\dot{W}_{max}$) and 4 min at 80% $\dot{W}_{max}$ (55/80) has been developed (Hough *et al.*, 2011). It was found that plasma and salivary cortisol and testosterone concentrations were increased in normally trained individuals, and therefore highlighting that these hormones elevate when not in an overreached state. The 55/80 protocol could possibly be used as a tool to identify and prevent overreaching and OTS occurring in athletes and this has been shown in a more recent study (Hough *et al.*, 2013). Using the same 55/80 protocol, a decreased exercise-induced salivary cortisol (11.1 to 3.1 $nmol \cdot L^{-1}$; 72% decrease), testosterone (407 to 258 $pmol \cdot L^{-1}$; 37% decrease) response and testosterone/cortisol ratio (12 to 4; 67% decrease) occurred following an 11 day endurance training period. The findings from this study suggest that overreached individuals (and possible individuals suffering from the OTS) have a blunted cortisol and testosterone response. Despite the development of a useful tool to highlight the hormonal alterations in overreached individuals, this may only be useful in a group familiar with cycling (i.e. cyclists). Therefore, it may be beneficial to develop a similar tool that would be applicable to runners.

The University of Bedfordshire laboratories has therefore developed a short-duration (30 min), high-intensity running test known as the $RPE_{treadmill}$. This running test consists of 30 min continuous running alternating between blocks of 1 min at 11 (fairly light) on the 6 – 20 Borg rating of perceived exertion (RPE) scale and 4 min at 15 (hard) on the Borg scale. This protocol has been shown to elicit reproducible exercise-induced elevations of plasma and salivary cortisol and testosterone when in a normal trained state (Leal, 2017). They reported a 42% elevation in plasma cortisol as well as a 64% elevation in salivary cortisol from pre- to post-exercise. They also found that plasma and salivary testosterone elevated by 42% from

pre- to post-exercise. This protocol could therefore highlight any blunted cortisol and testosterone responses that may occur when in an overreached state.

2.2.1 Cortisol and the Hypothalamic-Pituitary-Adrenal Axis

Stress is an innate response to survival in order to maintain homeostasis, thus the endocrine, nervous and immune systems are activated (Smith and Vale, 2006). The HPA axis is an essential component in responding to stressful stimuli which are internal or external in nature (Chrousos, 1995). The regulation of the HPA axis is dependent upon negative feedback mechanisms (Tsigos and Chrousos, 2002). The pathway originates with synthesis and secretion of corticotrophin-releasing hormone (CRH) from neurons within the paraventricular nuclei (PVN) of the hypothalamus (Chrousos, 1995). During a state of rest, the body continuously releases CRH into the hypophyseal portal system throughout the day with secretion episodes occurring two to three times per hour (Engler *et al.*, 1989). The hypophyseal portal system is a blood portal system consisting of blood vessels which links the hypothalamus to the pituitary gland (Capen, 2001). In response to stressors such as exercise, CRH production is significantly increased into the hypophyseal portal system that accesses the anterior pituitary gland. Within the anterior pituitary gland, CRH binds together to its respective cognate transmembrane receptors which trigger the release of ACTH and is secreted into circulatory system (Smith and Vale, 2006). ACTH travels and causes it's action in the adrenal glands (specifically the adrenal cortex) stimulating the secretion of cortisol located in the zona fasciculate (Smith and Vale, 2006; Nicolaides *et al.*, 2014). The zona fasciculate is one of three sections which make up the adrenal cortex; the other two are known as the zona glomerulosa and zona reticularis (Sayers, 1950).

Cortisol is the main glucocorticoid in humans which acts as the final hormonal product in the HPA axis pathway (Turnball and Rivier, 1999) where approximately 75% of total cortisol is bound to cortisol-binding globulin (CBG) and 15% to albumin within the blood (Kraemer and Ratamess, 2005). The remainder represents the biologically active fraction of free cortisol which is not bound to CBG (Duplessis *et al.*, 2010) and has been suggested to reflect the HPA reserve more accurately (Torpy and Ho, 2007). The biological active fraction enables cortisol to be readily available for tissue uptake (Pardridge, 1986). The unbound serum (free) cortisol is important as it enters the saliva via intracellular mechanisms. The majority of cortisol within the saliva remains free from bounding proteins.

Salivary cortisol has been shown to have positive correlations with serum cortisol at rest (Vining *et al.*, 1983; O'Connor and Corrigan, 1987; Lippi *et al.*, 2009; Duplessis *et al.*, 2010) and has been suggested as a reliable marker to use in response to exercise stress (O'Connor and Corrigan, 1987; Cadore *et al.*, 2008; Crewther *et al.*, 2010). An early study by O'Connor and Corrigan (1987) examined the salivary and serum cortisol response to 30 min of cycling at 75% $\dot{V}O_{2max}$. The authors reported a strong positive correlation between salivary and serum cortisol at rest (r = 0.89), immediately after exercise (r = 0.90) and 15 min post-exercise (r = 0.93).

Circulatory cortisol specifically aims to maintain a state of homeostasis during non-stressful and stressful environments by acting on peripheral organs via intracellular receptors throughout the body (Bamberger, Schulte and Chrousos, 1996). Actions of cortisol include energy metabolism, liver gluconeogenesis, enhances lipolysis, and influences blood pressure and electrolyte balances. Cortisol also acts upon physiological systems such as the immune system and the cardiovascular system (Kudielka and Kirschbaum, 2005).

2.2.2 Cortisol Responses to Acute Exercise

Cortisol can be increased following physical exercise (Davies and Few, 1973; Hill *et al.*, 2008). This increase in cortisol levels in response to exercise is dependent upon the intensity, duration, mode of exercise and training status (Meeusen *et al.*, 2013). A general consensus within the literature suggests that an increase in exercise intensity causes a proportional increase in cortisol concentrations, peaking 15-30 min post-exercise and returning to baseline levels within one hour (McKeever, 2002). In order for cortisol concentrations to increase, a specific exercise intensity threshold must be attained. Studies have established that the exercise must be moderate to high-intensity (>60% $\dot{V}O_{2max}$) lasting at least 20-30 min in duration to induce increases in circulating cortisol levels (Luger *et al.*, 1987; Brownlee, Moore and Hackney, 2005; Hill *et al.*, 2008). Hill *et al.* (2008) examined the plasma cortisol response to 30 min of exercise at intensities of 40, 60 and 80% of individuals $\dot{V}O_{2max}$ in 12 active moderately trained males. They observed a significant increase from pre-exercise values of 40% post-exercise at 60% $\dot{V}O_{2max}$ and an 83% increase post-exercise at 80% $\dot{V}O_{2max}$. Exercise-induced cortisol increases have also been shown to occur following short-duration, high-intensity exercise (~75% $\dot{V}O_{2max}$) in healthy males (Meeusen *et al.*, 2004;

Hough *et al.*, 2011, 2013). In comparison, exercise of lower intensities (40% $\dot{V}O_{2max}$) does not cause significant increases in cortisol with one study suggesting that circulatory cortisol levels is reduced once plasma volume reduction and circadian factors were corrected for (Hill *et al.*, 2008). However, it should not be assumed that cortisol responses to exercise will occur immediately with increases shown to occur at approximately four minutes (Port, 1991). Therefore, it is evident from previous research that the exercise intensity and duration is important to elicit significant cortisol responses when designing the exercise protocol.

The training status of the athlete can also have an influence on the cortisol response to acute exercise. Paccotti *et al.* (2005) investigated plasma and salivary cortisol responses to an acute bout of high-intensity isokinetic exercise in endurance trained athletes, power-trained athletes (n = 20) and non-competitive athletes (n = 11). They reported a 107% higher salivary cortisol response in the endurance and power-trained athletes compared to the non-competitive athletes immediately after exercise (22.1 ± 16.4 vs 10.7 ± 5.9 nmol·L^{-1}) as well as 90 min (29.2 ± 19.8 vs 17.1 ± 20 nmol·L^{-1}) and 120 min after exercise (22.2 ± 16.2 vs 11.7 ± 12.1 nmol·L^{-1}). This difference between endurance, power-trained and non-competitive athletes is supported by the moderate to large ES observed in this study which highlights the extent of varying training status can have on the cortisol response. A similar finding was also observed between competitive athletes (n = 20) and sedentary individuals (n = 10) after performing isokinetic exercise (Minetto *et al.*, 2007). It was reported that exercise-induced salivary cortisol response was 96% higher in competitive athletes (22.1 ± 16.4 nmol·L^{-1}) compared to sedentary individuals (11.3 ± 5.9 nmol·L^{-1}). A large ES was observed between competitive and non-competitive athletes. A summary of the cortisol responses to acute exercise is presented in Table 1.

2.2.3 Testosterone and the Hypothalamic-Pituitary-Gonadal axis

The HPG axis is also a major component of the endocrine system. Neurons located within the hypothalamus produce and secrete gonadotrophin-releasing hormone (GnRH) in response to stress and therefore activate the HPG axis (Kim, 2007). GnRH is then transported to the anterior pituitary gland via the hypothalamic hypophyseal portal vein where GnRH stimulates the production of luteinizing hormone (LH) from the gonadotrophs (Kim, 2007). Gonadotrophs are a sub-type of basophil cells found in the medial section of anterior pituitary gland which secrete hormones (specifically LH) and act upon the reproductive organs

(Hinson, Raven and Chew, 2010). The anterior pituitary gland releases LH into circulation and is taken up by the gonads stimulating the release of testosterone. Explicitly within the gonads, LH is responsible for the testosterone production in the Leydig cells of males (Vingren *et al.*, 2012). LH is processed further as it binds to G-protein-coupled membrane receptor, activating cyclic adenosine monophosphate-dependent protein kinases (protein kinase A) which is responsible for the stimulating the rate-limiting step in the synthesis of testosterone (Miller, 1988). Moreover, testosterone cannot be stored in the cells that produce it due to being anabolic in nature and therefore it is released instantly from the cells following production (Vingren *et al.*, 2012). Similar to cortisol, testosterone is regulated by a negative feedback loop and the central nervous system (CNS). The negative feedback loop acts by inhibiting GnRH release via hypothalamus and signalling the gonads to inhibit LH response to GnRH (Hinson, Raven and Chew, 2010).

Once testosterone has been released into circulation, the majority of it is bound to binding proteins that are hydrophilic due its inability to dissolve into the blood easily (Vingren *et al.*, 2012). Within plasma, testosterone specifically binds to sex hormone-binding globulin (SHBG) (44-60%) and non-specifically albumin (38-54%) which represents the total fraction of testosterone (Cumming and Wali, 1985; Hayes, Bickerstaff and Baker, 2010). The remainder (0.2-2%) is the free fraction of testosterone which is unbound within plasma, however, it is the most biologically active fraction of testosterone (Kraemer *et al.*, 1998). Albumin-bound testosterone is also biologically active which means that both are readily available for tissue uptake compared to testosterone bound to SHBG (Pardridge, 1986; Hayes, Bickerstaff and Baker, 2010). Therefore, free and albumin-bound testosterone has been suggested to be a better marker of total testosterone in plasma (Hayes, Bickerstaff and Baker, 2010). Testosterone is not just limited to plasma and can be diffused into salivary glands through intracellular mechanisms with research suggesting that the majority of testosterone which is diffused into salivary glands is limited to the free fraction of testosterone (Rilling *et al.*, 1996).

Several studies have shown a very strong, positive correlation (r = 0.70-0.97) between blood and saliva in males at rest (Wang *et al.*, 1981; Sannikka *et al.*, 1983; Vittek *et al.*, 1985; Shirtcliff, Granger and Likos, 2002) and therefore salivary testosterone levels can be used as an index of free serum testosterone. Vittek *et al.* (1985) examined the relationship between salivary and serum free and total testosterone concentrations in 45 healthy individuals and found a significant positive relationship between salivary and serum free testosterone

concentrations (r = 0.97). The exercise-induced salivary testosterone responses have been shown to have a positive correlation with the exercise-induced serum testosterone levels (Crewther *et al.*, 2010).

Testosterone is an androgen, steroid hormone produced from cholesterol (Vingren *et al.*, 2012). It is responsible for exerting anabolic actions upon muscle tissue, specifically to increase protein synthesis and decrease protein degradation to enhance muscle growth (Loebel and Kraemer, 1998). Testosterone also influences the development of male reproductive system, cardiovascular system and immune system (Vingren *et al.*, 2012).

2.2.4 Testosterone Responses to Acute Exercise

Testosterone responses to acute exercise are of similar nature to cortisol in which testosterone increases linearly to the exercise intensity (Wilkerson, Horvath and Gutin, 1980). However, peak concentrations of total and free testosterone occur at the end of the exercise bout and are expected to return to, or below, baseline within 30 min of exercise (Kvorning *et al.*, 2007). It has been shown that circulatory levels of total and free testosterone increase instantly following resistance and endurance exercise (Cadore *et al.*, 2008; McCaulley *et al.*, 2009; Hough *et al.*, 2011). Hough *et al.* (2011) examined plasma and salivary testosterone responses to three endurance-based cycling bouts (continuous cycle to fatigue, 30 min cycling alternating every 1 min at 60% $\dot{W}_{max}$ and 90% $\dot{W}_{max}$ [60/90] and 30 min 55/80 cycling bout) and resistance exercise (squatting 8 sets of 10 reps at 10 rep max) in ten healthy males. Hough and colleagues reported plasma and salivary testosterone responses to significantly increase by ~60-120% (cortisol) and ~35-82% (testosterone) following all exercise trials with peak concentrations occurring at 0 and ~10 min post-exercise. Furthermore, plasma and salivary testosterone levels were shown to return to pre-exercise levels at 30 min post-exercise in the cycle to fatigue and 60/90 trial and 40 min post-exercise in the 50/80 trial. Additionally, similar findings have been reported in a study by Collomp *et al.* (2008) who found that plasma-free testosterone responses increased by 66% following a 30 min cycle at 75% $\dot{V}O_{2max}$ in eight recreational male athletes. A summary of the testosterone responses to acute exercise are presented in Table 1.

Table 1 Overview of the salivary cortisol and testosterone responses to acute exercise.

Author	Participants	Exercise	Exercise duration	Cortisol response	Testosterone response
Collomp *et al.* (2008)	8 recreational male athletes	Cycle at 75% $\dot{V}O_{2max}$	30 min	N/A	66% increase
Hill *et al.* (2008)	12 actively moderately trained males	Treadmill running at 40, 60 and 80% $\dot{V}O_{2max}$	30 min	40% increase at 60% $\dot{V}O_{2max}$ and 86% increase at 80% $\dot{V}O_{2max}$	N/A
Hough *et al.* (2011)	10 healthy males	Cycling at ~75% $\dot{W}_{max}$	30 min	120% increase	35% increase
McCaulley *et al.* (2009)	10 males	Resistance exercise – 4 sets of 10 squats at 75% 1 rep max	~15 min	12% increase	32% increase
Meeusen *et al.* (2004)	7 well-trained male cyclists	Graded exercise incremental test until exhaustion	~23 min	32% increase	N/A
Minetto *et al.* (2007)	20 competitive athletes and 10 sedentary individuals	Isokinetic exercise (total of 160 isokinetic contractions)	15 min	96% increase	N/A
Paccotti *et al.* (2005)	20 competitive endurance and power-trained male athletes	High-intensity isokinetic exercise (total of 160 maximal contractions of knee flexor and extensor muscle groups)	15 min	77% increase	N/A
Leal *et al.* (2017)	10 active healthy active males	High-intensity running test (1 min at 11 RPE and 4 min 15 RPE)	30 min	64% increase	42% increase

2.2.5 Cortisol and Testosterone Responses to Intensified Training Periods

Studies examining the resting and exercise-induced cortisol and testosterone responses to periods of intensified training have shown contrasting findings with increases, decreases and no changes being reported. A longitudinal study by Filaire *et al.* (2004) investigated the resting hormonal responses of 12 national cyclists where the training load was progressively increased over a period of 8 months. They found that no significant salivary cortisol and testosterone changes occurred. The resting hormonal measurements to shorter periods of intensified training have also been shown to be equivocal. Ishigaki *et al.* (2005) examined the relationship between resting plasma cortisol and testosterone levels in 13 collegiate male distance runners before and after an eight day training camp. The authors reported a significant increase (42%) in plasma cortisol concentrations (pre-training: 326.2 ± 55.2; post-training: 463.1 ± 110.1 $nmol \cdot L^{-1}$) and a significant decrease (35%) in plasma testosterone concentrations (pre-training: 14.2 ± 4.4; post-training: 9.2 ± 3.4 $pmol \cdot L^{-1}$). Slivka *et al.* (2010) reported that salivary cortisol and testosterone did not change in eight trained cyclists over a period of 21 days of intensified training where volume and intensity increased by 418% of normal training period. In contrast, examining the exercise-induced hormone responses to periods of intensified training have produced more consistent findings to identify overreached athletes or athletes who may be at risk of developing the OTS (Meeusen *et al.*, 2004, 2010; Hough *et al.*, 2013; Hough, Robertson and Gleeson, 2015).

As detailed previously, Meeusen *et al.* (2004) found blunted exercise-induced plasma cortisol responses following 10 day training camp between overreached and normally-trained individuals. Furthermore, Hough *et al.* (2013) observed a blunted salivary exercise-induced cortisol and testosterone response following an 11 day intensified training period. Blunted salivary exercise-induced testosterone responses have also been shown to occur in elite male triathletes (Hough, Robertson and Gleeson, 2015). Using the same 55/80 cycling protocol from previous studies, Hough and colleagues reported an exercise-induced salivary testosterone decrease from 114% to 85% following a 10 day training camp (where volume and intensity of the exercise were elevated). However, salivary cortisol responses were unaltered from pre- to post-training. This could suggest that testosterone may be a better marker of overreaching, specifically in elite athletes. A summary of the salivary cortisol and testosterone responses to intensified training periods are presented in Table 2.

Table 2 Overview of the salivary cortisol and testosterone responses to intensified training periods.

Author	Participants	Training duration	Sample type (resting or exercise)	Cortisol response	Testosterone response
Filaire *et al.* (2004)	12 elite national male cyclists	8 months	Rest	No change	No change
Flynn *et al.* (1994)	8 male cross-country runners	6 weeks	Rest	No change	No change
Hooper *et al.* (1995)	14 male and female swimmers	6 months	Rest	No change	No change
Hough *et al.* (2013)	12 recreationally active males	11 days	Exercise	72% decrease	37% decrease
Hough *et al.* (2015)	7 elite male triathletes	10 days	Exercise	No change	25% decrease
Ishigaki *et al.* (2005)	13 male collegiate runners	8 days	Rest	42% increase	35% decrease
Meeusen *et al.* (2004)	7 well-trained male cyclists	10 days	Exercise	113% decrease	N/A
Slivka *et al.* (2010)	8 male cyclists	21 days	Exercise	No change	No change
Urhausen *et al.* (1998)	17 male endurance athletes	19 months	Rest	No change	No change

2.3 Markers of Overreaching – Immune Responses

Given that overreaching is a result of intensified training combined with inadequate recovery, this could increase the duration of the "open-window" leading to immunosuppression (Halson and Jeukendrup, 2004). It has been suggested that endurance athletes during periods overreaching/overtraining and competition experience illnesses more frequently, especially URS (Nieman, 2003). It has been suggested that URS are viral in nature, however, the key immunological mechanisms which underpin the increased occurrence of URS in athletes compared with non-athletes still remains unclear within the literature (Gleeson and Bishop, 2013). Although the exact mechanisms cannot be explained, a relationship between exercise intensity/volume and susceptibility to the URS does exist and may be modelled in the form of a J-shaped curve (Nieman, 1994b, 1994a). The J-shaped curve model suggests that exercise intensity/volume of moderate levels reduces the risk of URS to below average compared to individuals who perform sedentary levels of exercise. However, individuals who perform periods of high-intensity exercise or periods of intensified training increase the risk of URS to levels above average.

The importance of the immune system for defence against pathogenic (disease-causing) micro-organisms including bacteria, protozoa, viruses and fungi is essential in ensuring that the homeostasis of the human body is maintained (Gleeson, Bishop and Walsh, 2013). Although, hormonal alterations could be indicative of overreaching and the OTS, the literature also suggests that immunological responses could also help identify and prevent these states from occurring (Halson and Jeukendrup, 2004; Meeusen *et al.*, 2013; Kreher, 2016). Growing interest into the immune system as a potential indicator of overreaching is due to its association with the endocrine system. Given that stress (exercise) activates the HPA axis, which in turn releases cortisol, it has been suggested to act upon the immune system by either being immunomodulatory or immunosuppressive (Dhabhar, 2009). Naturally produced cortisol has been shown to be immunomodulatory whereas high levels of cortisol has been shown to be immunosuppressive (Dhabhar, 2009). Walsh *et al.* (2011) suggests that chronic exercise/periods of intensified training induce elevations in cortisol production which could attribute to the down-regulation of IgA synthesis and polymeric immunoglobulin receptor (pIgR) expression resulting in the IgA response to be suppressed. IgA is one of the primary antibodies within mucosal immunity and is produced via mature B cells located in the blood and acts as a defence mechanism against pathogens in the oral and nasal cavities (Gleeson, 2000; Gleeson and Pyne, 2000). One study has examined the

influence of a single synthetic glucocorticoid dexamethasone injection on salivary IgA concentrations and secretions and they found that salivary IgA decreased 24 h after the injection (Wira, Sandoe and Steele, 1990). Therefore, during periods of intensified training, cortisol could supress the synthesis of IgA by B cells in the submucosa (Saxon *et al.*, 1978). However, the mechanisms which underpin the exercise-induced SIgA responses still remains unclear and more studies need to be undertaken to clarify them (Walsh *et al.*, 2011).

Specifically within the mucosal immune system, SIgA has been suggested as marker of overreaching and the OTS (MacKinnon, 2000). Tomasi *et al.* (1982) were the first to examine the mucosal immune responses to exercise, suggesting that individuals were more susceptible to viral and bacterial infections following intense exercise due to deficiency on the mucosal surface. This speculation was after observing a 20% decrease in SIgA concentrations in elite cross-country skiers compared with recreational athletes following two to three hours of competition. Since then a number of studies have been published investigating the mucosal immune responses to acute exercise and periods of intensified training.

2.3.1 Immunoglobulin A

The mucosal immune system is an important component of the acquired immune system as it provides the first line of defence against potential pathogens invading the oral and nasal cavities (Gleeson and Pyne, 2000). The primary antibody of the mucosal immune system is IgA which is a glycoprotein produced by mature B cells in the blood and secreted into bodily fluids such as saliva, tears, nasopharyngeal, bronchial, intestinal and urogenital secretions (Gleeson, 2000). As the primary antibody, IgA has a number of functions to fulfil across the mucosal surfaces such as the linings of the respiratory, gastrointestinal and genitourinary tracts (Woof and Ken, 2006).

IgA consists of two isotypes known as IgA1 and IgA2 (Gleeson and Pyne, 2000). Each isotype distributes itself depending on the mucosal site, for example, the majority of IgA1 is in the salivary glands (60-80%) and the nasal-associated lymphoid tissue (90%) (Gleeson and Pyne, 2000). Whereas, the majority of IgA2 is in the distal gastrointestinal tract (60%) (Gleeson and Pyne, 2000). Initially, IgA in its monomeric form is secreted by the B cells (plasma cells) and forms dimeric IgA when two IgA monomers are joined together by a small polypeptide structure called the J-chain (Tsujita and Morimoto, 1999). Dimeric IgA

molecules diffuse through the basement membranes to bind to the pIgR located on the basolateral surfaces of the epithelial cell (Bishop and Gleeson, 2009). The binding process to pIgA enables transcytosis of IgA across the mucosal epithelium where pIgR is proteolytically cleaved at the apical surface which leaves the secretory component bound to the dimeric IgA molecule and therefore forming secretory IgA (Lamm, 1998; Bishop and Gleeson, 2009). The secretory component of IgA is essential to the stability of IgA and prevents degradation by bacterial and digestive enzymes in secretory fluids such as saliva (Johansen, Braathen and Brandtzaeg, 2001). This process fulfils three potential mechanisms in which SIgA protects against microbial pathogens: through prevention of pathogen adherence and penetration of the mucosal epithelium, by neutralising viruses within the epithelial cells during transcytosis and by removal of locally formed immune complexes across mucosal epithelial cells to the luminal surface (Lamm, 1998).

2.3.2 Salivary Secretory IgA Responses to Acute Exercise

The association between acute bouts of exercise and the SIgA response is largely dependent upon the intensity, duration and the mode of exercise (Walsh *et al.*, 2011). High-intensity exercise has been shown to decrease SIgA concentrations in many studies with levels returning to baseline within 1 h of exercise completion (Tomasi *et al.*, 1982; MacKinnon and Jenkins, 1993; Nieman *et al.*, 2002; Walsh *et al.*, 2002). However, some studies have reported increases (Dorrington, Gleeson and Callister, 2003; Sari-Sarraf *et al.*, 2007) and no changes occurring at all (Mackinnon and Hooper, 1994). Moderate to low-intensity (<60% $\dot{V}O_{2max}$) aerobic exercise lasting less than one hour have minimal impact on SIgA concentrations (Bishop and Gleeson, 2009) and SIgA concentrations are generally unchanged following resistance exercise (Koch, 2010) and moderate to high-intensity intermittent/interval exercise (Bishop et al 1999, Davison 2011). Mackinnon *et al.* (1989) examined salivary IgA responses to 2 h of cycling at 70-75% $\dot{V}O_{2max}$ in 8 male cyclists. They found that salivary IgA concentrations decreased post-exercise by 63% and remained at this level for one hour post-exercise with levels returning to baseline within 24 h of exercise. Further studies have been conducted on endurance exercise and have shown salivary IgA secretion rate to significantly decrease by 34% following competitive marathon running (Nieman *et al.*, 2002) and ultramarathon running (Palmer *et al.*, 2003). To the contrary, Walsh *et al.* (2002) has shown a 17% increase in SIgA concentrations in well-trained cyclists

following 2 h cycling at 70% $\dot{V}O_{2max}$. Comparable findings have also been found by Laing *et al.* (2005) who reported a 21% increase in salivary IgA concentrations (pre-exercise: 91 ± 12; post-exercise: 110 ± 13 $mg \cdot L^{-1}$) following 2 h cycling at 65% $\dot{V}O_{2max}$. Overall, the majority of the literature suggests that long-duration exercise has a negative impact on SIgA responses. However, limited amount of research has been conducted on the impact of SIgA responses to short-duration, high-intensity exercise (Allgrove *et al.*, 2008).

Short-duration (~30 min), high-intensity exercise has been shown to increase SIgA concentrations and secretion rates (Blannin *et al.*, 1998; Allgrove *et al.*, 2008). An early study examined the effect of cycling to exhaustion at different intensities (55% and 80% $\dot{V}O_{2max}$) on salivary IgA concentrations and secretion rate (Blannin *et al.*, 1998). They found that salivary IgA concentrations (pre-exercise: 88 ± 11; post-exercise: 265 ± 34 $mg \cdot L^{-1}$) and secretion rate (pre-exercise: 18.9 ± 3.5; post-exercise: 29.9 ± 4.6 $\mu g \cdot min^{-1}$) significantly increased by 201% and 58% following an 80% $\dot{V}O_{2max}$ cycle to exhaustion test (~37 min). Moreover, Allgrove and colleagues investigated the effects of different exercise intensities (50% $\dot{V}O_{2max}$, 75% $\dot{V}O_{2max}$ and incremental test to exhaustion) in 10 healthy active males and they reported a significant increase in salivary IgA concentrations (56%) and secretion rates (50%) post-exercise during incremental test to exhaustion only. This study highlights that short-duration, high-intensity exercise induces an increase in the SIgA response. However, it is clear that SIgA responses to acute exercise still remains inconsistent due to the difference in protocols, collection methods used (stimulated vs unstimulated) and how SIgA is expressed (i.e. absolute concentration or as a secretion rate) (Walsh *et al.*, 2011). A summary of the SIgA responses to acute exercise are presented in Table 3.

Table 3 Overview of the SIgA responses to acute exercise.

Author	Participants	Exercise	Exercise duration	IgA expression	IgA response
Allgrove *et al.* (2008)	10 healthy active males	Incremental cycle test to exhaustion	~22 min	Absolute concentration Secretion rate	56% increase 50% increase
Blannin *et al.* (1998)	18 healthy active males	Cycle to exhaustion at 80% $\dot{V}O_{2max}$	~37 min	Absolute concentration Secretion rate	201% increase 58% increase
Laing *et al.* (2005)	13 healthy males	Cycle at 65% $\dot{V}O_{2max}$	2 h	Absolute concentration Secretion rate	15% increase 35% decrease
Mackinnon and Hooper (1994)	18 recreational-competitive male and female endurance athletes	Treadmill run at 55% and 75% $\dot{V}O_{2max}$	40-90 min	Secretion rate	No change
Mackinnon and Jenkins (1993)	12 healthy active males	Five 60 s bouts of supramaximal interval exercise at 0.075 $g \cdot kg^{-1}$ body mass on the cycle ergometer	5 min	Absolute concentration Flow rate	19% increase 50-65% decrease
Mackinnon *et al.* (1989)	8 male cyclists	Cycle at 70-75% $\dot{V}O_{2max}$	2 h	Absolute concentration	63% decrease
Nieman *et al.* (2002)	98 male and female runners	Competitive marathon race	~4.5 h	Absolute concentration Secretion rate	19% decrease 34% decrease
Palmer *et al.* (2003)	28 ultramarathon male and female runners	Ultramarathon 80 km race	5-12 h	Absolute concentration Secretion rate	27% decrease 45% decrease
Sari-Sarraf *et al.* (2007)	10 healthy active males	Soccer-specific intermittent exercise	90 min	Absolute concentration Secretion rate	186% increase 128% increase
Tomasi *et al.* (1982)	Elite and recreational	Cross-country skiing race	2-3 h	Absolute concentration	20% decrease

	cross country skiers				
Walsh *et al.* (2002)	15 trained male cyclists	Cycle at 70% $\dot{V}O_{2max}$	2 h	Absolute concentration	17% increase
				Secretion rate	20% decrease
				Flow rate	31% decrease

2.3.3 Salivary Secretory IgA Responses to Intensified Training Periods

To date, an abundant amount of research has been conducted on SIgA responses to long-term training (>1 month) with many studies showing a negative relationship between SIgA responses (decrease) and URS (increased incidence). Many of these studies have measured resting SIgA responses before, during and after a competitive season. In an early and much cited study by Gleeson *et al*. (1995, 1999), a 10% decrease in salivary IgA concentrations were observed after individual training sessions in elite swimmers whereas salivary IgA concentrations increased by 11% in controls. Furthermore, salivary IgA concentrations were found to decrease over a 7 month period in both pre- and post-exercise samples. The findings from this study suggest daily intense exercise sessions over a long period of time supress the mucosal immune function. A number of long-term studies have also found similar results that have been examined in elite swimmers (Tharp and Barnes, 1990), American college football players (Fahlman and Engels, 2005) and elite yacht players (Neville, Gleeson and Folland, 2008).

Contrary to these findings, Mackinnon and Hooper (1994) reported no significant changes in resting salivary IgA over a 6 month competitive season in 14 elite swimmers. Although, they did observe that salivary IgA concentrations were consistently lower by 18-32% in stale athletes and possibly predisposed to OTS (defined as a failure to improve performance from early season and persistent fatigue) compared with well-trained swimmers (n = 3). Therefore, suggesting that mucosal immunity is suppressed further in overreached and OTS athletes. In a more recent study, symptoms of URS were monitored during a 4 month period of winter training to assess the function of the immune system in 80 endurance athletes (ranging from recreational to Olympic level) (Gleeson *et al.*, 2012). Gleeson and colleagues found that athletes with symptoms of URS did not have significantly different resting salivary IgA concentrations compared with athletes with no URS (145 ± 79 vs 155 ± 95 $mg \cdot L^{-1}$). Although they did find that resting salivary IgA secretion rate (49.3 ± 35.8 vs 80.3 ± 52.5 $\mu g \cdot min^{-1}$) and flow rate (0.35 ± 0.19 vs 0.53 ± 0.24 $mL \cdot min^{-1}$) were significantly lower in athletes with URS compared to those with no symptoms. Furthermore, one study showed no significant changes in the resting salivary IgA samples in 41 elite swimmers over a 5 month period leading up to the 1998 Olympic Games (Pyne *et al.*, 2001).

Short-term training studies have also shown comparable results to long-term training studies. Studies designed to intensify the training load to induce a state of overreaching have also

shown to decrease salivary IgA responses from pre- to post-training (Mackinnon, 1996; Robson *et al.*, 1999; Gleeson, Ginn and Francis, 2000; Gleeson *et al.*, 2002; Libicz *et al.*, 2006). A 67% decreased in resting concentrations of salivary IgA immediately before the onset of URS symptoms were found during a 30 day period of intensified training (load) in a group of elite male swimmers (Gleeson *et al.*, 2002). A similar finding has been reported in one male elite kayaker in which salivary IgA response decreased by 69% following a 14 day intensified training period (Gleeson, Ginn and Francis, 2000). A criticism of this study would be that only one participant was used. A study by Robson *et al.* (1999) found that resting salivary IgA concentrations fell by ~10% in endurance trained male runners following 2-3 weeks of intensified training. Furthermore, suppressed levels of salivary IgA in terms of concentration, secretion rate and flow rate were also shown to occur in elite male triathletes during the 6 day French Iron Man Tour (Libicz *et al.*, 2006). Over the course of the 6 days, salivary IgA secretion rate fell by 52%.

Several studies have also examined the mucosal responses to periods of military training which gives a further understanding of the responses expected to happen during periods of intensified training which can mean many of these individuals are in a overreached/overtrained state (Carins and Booth, 2002; Gomez-Merino *et al.*, 2005; Tiollier *et al.*, 2005). A study using 29 military recruits used SIgA as marker to assess mucosal immunity during a 19 day Royal Air Force survival course (Carins and Booth, 2002). They found a negative relationship between SIgA and the occurrence of URS. Moreover, Tiollier *et al.* (2005) examined salivary IgA responses to 3 weeks of military training followed by a 5 day combat course and reported no changes in salivary IgA concentrations following 3 weeks of military training. Although no changes were observed following the 3 weeks of training, Tiollier and colleagues found a ~41% decrease after the 5 day combat course. These studies highlight how intensified periods of training have a damaging effect on SIgA responses which in turn leads to higher incidences of URS. Although military studies provide a useful insight, exercise alone may not be the sole contributor to the responses observed as many of these studies involve other stressors such as dietary energy deficiencies, sleep deprivation and psychological challenges. These stressors could therefore influence the immuno-endocrine responses and as a result increasing the exercise-induced alterations (Oliver *et al.*, 2007).

Much of literature suggests that both long-term and short-term periods of intensified training have a profound impact on the mucosal immunity. Many studies have shown resting levels of

SIgA concentrations and secretion rates to be suppressed from pre- to post-training which may be a predisposition to high URS susceptibility in athletes (Walsh and Oliver, 2016). However, there is still a lack of literature surrounding the exercise-induced SIgA responses to periods of intensified training. A summary of the SIgA responses to intensified training periods are presented in Table 4.

Table 4 Overview of the SIgA responses to intensified training periods.

Author	Participants	Training duration	Sample type (resting or exercise)	IgA expression	IgA response
Carins and Booth (2002)	29 Australian Air Force	19 days	Rest	Absolute concentration	33% decrease
Fahlman and Engels (2005)	100 male American college football players	12 months	Rest	Absolute concentration Secretion rate	38% decrease 30% decrease
Gleeson *et al.* (1995)	26 elite swimmers	7 months	Exercise	Absolute concentration	10% decrease
Gleeson *et al.* (2000)	1 elite male kayaker	14 days	Exercise	Absolute concentration	69% decrease
Gleeson *et al.* (2002)	14 elite male swimmers	30 days	Rest	Absolute concentration	67% decrease
Gleeson *et al.* (2012)	80 male and female endurance athletes	4 months	Rest	Absolute concentration Secretion rate Flow rate	No change 39% decrease 33% decrease
Libicz *et al.* (2006)	8 elite male triathletes	6 days	Exercise	Absolute concentration Flow rate Secretion rate	38% decrease 56% decrease 52% decrease
Mackinnon and Hooper (1994)	14 elite swimmers	6 months	Rest	Absolute concentration	18-32% decrease (stale athletes)

Neville *et al.* (2008)	38 elite yacht racing athletes	50 weeks	Rest	Absolute concentration	28% decrease (3 weeks prior to URS episodes)
Pyne *et al.* (2001)	21 elite male swimmers	5 months	Rest	Absolute concentration	No change
Robson *et al.* (1999)	8 endurance trained male runners	2-3 weeks	Rest	Absolute concentration	10% decrease
Tharpe and Barnes (1990)	21 elite male swimmers	3 months	Exercise	Absolute concentration	53% decrease
Tiollier *et al.* (2005)	21 male military cadets	4 weeks	Rest	Absolute concentration	No change (3 weeks training) 41% decrease (5 day combat course)

2.4 Circadian Rhythm

It well established within the literature that cortisol and testosterone are hormones regulated via a circadian rhythm and is one of the most distinctive within human physiology (Hill *et al.*, 2008). It is understood that circadian rhythms are biological variations which are repetitive in nature and occur approximately over a 24 h period (Reilly and Waterhouse, 2009). Research has suggested that under natural conditions, circadian rhythms are self-sustaining endogenous biological timekeepers due to their ability to be synchronised with the external world (Mrosovsky *et al.*, 1989; Pittendrigh, 1993). As a result, individuals can anticipate and prepare for challenges which arise during the day and night (Hastings, O'Neill and Maywood, 2007). The circadian rhythm of cortisol and testosterone is regulated by the main circadian oscillator known as the suprachiasmatic nucleus (SCN) (also referred to as the circadian pacemaker or "biologic clock") which is located in the anterior hypothalamus (Chan and Debono, 2010).

Healthy individuals experience very low levels of cortisol at midnight that increase overnight with levels peaking in the morning 30 min after awakening (Hayes, Bickerstaff and Baker, 2010). Thereafter, a slow decline in cortisol levels occur throughout the day (Debono *et al.*, 2009). This steady rise in cortisol overnight is believed to be the body anticipating stresses associated with wakefulness and increased activity (Hastings, O'Neill and Maywood, 2007). This phenomenon is known as the cortisol awakening response (CAR) (Fries, Dettenborn and Kirschbaum, 2009). Factors such as night-time work and daytime sleep have been shown to change cortisol's 24 h cycle by altering sleep-wake rhythm (Czeisler *et al.*, 1990). Normal salivary cortisol concentrations range from ~4-15 $n \cdot mol^{-1}$ (Laudat *et al.*, 1988).

The same circadian rhythmicity also occurs with testosterone (Hayes, Bickerstaff and Baker, 2010). Levels of testosterone begin to rise at night, peak early morning (between 06:00 and 08:00 h) and then fall throughout the day (Constantini and Hackney, 2013). Studies have shown testosterone concentrations to decline by 42% from 06:00 h (awakening) to 23:00 h (Krieger *et al.*, 1971). Normal salivary testosterone concentrations range from ~400-700 $pmol \cdot L^{-1}$ (Kiess *et al.*, 1995; Kraemer *et al.*, 2001).

A circadian rhythm has also been reported in SIgA. In contrast to cortisol and testosterone, SIgA has been shown to display a different circadian pattern with levels peaking immediately upon awakening and then steadily falling throughout the day (Dimitriou, Sharp and Doherty,

2002; Shirakawa, Mitome and Oguchi, 2004). Although circadian patterns are different, SIgA levels are generally higher in the morning compared to the afternoon. Normal SIgA concentrations are ~100 $mg \cdot L^{-1}$ (Jafarzadeh *et al.*, 2010).

2.5 Markers of Overreaching – Performance Testing

A hallmark feature of overreaching and OTS is the decrease in sport-specific performance due to inability to sustain the exercise intensity during increases in training load (Urhausen, Gabriel and Kindermann, 1995; Meeusen *et al.*, 2004). In normal circumstances, athletes in a healthy state are able to complete the training programme prescribed to them. However, in cases where athletes are diagnosed with OTS, they are capable of starting the training programme or a race at normal pace but are incapable of completing the training load given to them or race as usual (Meeusen *et al.*, 2013). Therefore, it has been suggested that an exercise performance test is considered to be essential for the diagnosis of OTS (Halson and Jeukendrup, 2004).

In order to detect changes in performance in overreached and OTS athletes, the type of performance test and the intensity/duration need to be carefully considered as the magnitude of performance decline may vary (Halson and Jeukendrup, 2004). Therefore, a general consensus on which test is the most suitable still remains inconclusive within the literature. A number of performance tests exist, and these include time-to-fatigue, incremental and time trial (TT) tests. The importance of performance testing has been highlighted in one study investigating athletes with persistent fatigue (Reid *et al.*, 2004). They found that in 41 competitive athletes, 93% of them reported decreases in performance.

Time-to-fatigue tests have been shown to highlight larger reductions in exercise capacity in overreached and OTS athletes or in response to intensified training periods (Fry *et al.*, 1994; Meeusen *et al.*, 2004). An early study examined the effects of a 10 day intensified training period (included two daily intensive training sessions) on running performance in five well-trained males (Fry *et al.*, 1994). They found that time-to-fatigue (18 $km \cdot h^{-1}$ and 1% gradient on a treadmill) was 29% slower post-training compared to pre-training. Furthermore, Urhausen *et al.* (1998) reported a 27% decrease in time-to-fatigue in overtrained athletes (22.7 ± 2.3 min) compared to athletes in a normal trained state (16.6 ± 1.3 min). Although larger reductions in performance are reported compared to other methods of testing, they are

not an accurate performance indicator as do not sufficiently reflect the true demands of athletic competition (Halson and Jeukendrup, 2004).

Time trial tests have also shown decreases in performance (Jeukendrup *et al.*, 1992; Halson *et al.*, 2002, 2003). The benefits of using TT in studies is that they accurately reflect the sport-specific task of most sports and allow detection of subtle performance decrements compared to time-to-fatigue and incremental tests (Meeusen *et al.*, 2013). Jeukendrup and colleagues conducted a study investigating the impact of a 2 week intensified training period on cycling performance (outdoor 8.5 km TT) in seven male competitive cyclists. They reported a 6% decrease in cycling performance from pre- (13.8 ± 0.2 min) to post-training (14.5 ± 0.3 min). The same study also reported 3-4% decrease in maximal aerobic power during a graded incremental cycle test. In addition, a study by Halson *et al.* (2002) found a 10% decrease in TT performance (59.4 ± 1.9 vs 65.3 ± 2.6 min) and a 6% decrease in maximal power output (338 ± 17 vs 319 ± 17 W) following a 6 week intensified training period. These studies have highlighted that despite the variations in the type of performance tests used, a decline in exercise performance is evident in overreached individuals. Furthermore, the use of TT in overreaching studies enables other variables to be measured such as substrate kinetics, hormonal responses and sub-maximal measures (Meeusen *et al.*, 2013).

2.6 Aims and Hypotheses

The aim of the present study was to determine if there were any hormonal alterations of salivary cortisol and testosterone and mucosal immunological responses of SIgA to a 30 min high-intensity running bout before and after a 12 day intensified training period.

The hypotheses for the present study were as follows:

1. It is hypothesised that a decrease in exercise-induced salivary cortisol and testosterone response will be observed following a period of 12 days intensified training.
2. It is hypothesised that a decrease in exercise-induced SIgA response will be observed following a period of 12 days intensified training.
3. It is hypothesised that a negative relationship between SIgA response and URS incidences will occur following a period of 12 days intensified training.

4. It is hypothesised that the exercise-induced salivary cortisol, testosterone and SIgA responses will return to, or increase above pre-training levels following a 4 day recovery period.

2.7 Contributions

This MSc by Research study was conducted as collaborative effort with Diogo Leal who also submitted this as part of his PhD (Leal, 2017). Throughout the study, Diogo and I shared the responsibilities in order to complete the study on time. I attended all preliminary testing sessions, all main trials and all training days for each participant. During these sessions, I collected saliva samples from each participant and I later analysed them in the wet laboratory. I also prepared the participants for exercise (i.e. fitting HR monitors), performed the tests (i.e. $\dot{V}O_{2max}$ test), monitored each participant to ensure each test protocol were performed correctly and I also collected the necessary data needed for the study. Moreover, I was involved with participant recruitment and salivary analysis via ELISA method which was completed in partnership with Diogo.

CHAPTER 3 Methods

3.1 Power Analyses

Post-hoc power analyses were conducted with G*Power software version 3.1 (Heinrich University, Düsseldorf, Germany). The observed power for salivary cortisol, testosterone and SIgA was 0.550, 0.694 and 0.637, respectively.

3.2 Participants

Eight active, healthy males volunteered to participate in this study (participant physical and physiological characteristics are outlined in Table 5). All experimental procedures were approved by the University of Bedfordshire's Research Ethics Committee. An information sheet (appendix a) with a full explanation of the study, possible risks and benefits involved was given to each participant before testing commenced. On arrival, each participant completed an informed consent form (appendix b), physical activity readiness questionnaire (PAR-Q) (appendix c) and a health questionnaire (appendix d).

Table 5 Participant physical and physiological characteristics.

Variable	
Age (years)	21 ± 5
Height (cm)	178.2 ± 5.2
Body mass (kg)	72.8 ± 6.6
HR_{max} (beats·min^{-1})	192 ± 9
$v\dot{V}O_{2max}$ (km·h^{-1})	16.6 ± 2.2

Values are mean ± *SD*.

3.3 Inclusion and Exclusion Criteria

Prior to the commencement of the study, each participant completed an informed consent form, a PAR-Q and a health questionnaire. If participants answered "yes" to the any of the PAR-Q questions then they were excluded from the study. Furthermore, participants were required to be physically active in which they participated in at least 3 h of exercise per week

(Department of Health Physical Activity Health Improvement and Protection, 2011). Night shift workers were excluded from the present study as adjustment of their circadian rhythms takes at least 4 days (Niu *et al.*, 2015). Any participant carrying an injury was also excluded from the present study.

3.4 Experimental Procedures

All main trials took place at the University of Bedfordshire's Sport and Exercise Science Laboratories. For the main trials, each participant completed the $RPE_{treadmill}$ bout. This bout consisted of a 30 min self-paced continuous run with alternating blocks of 1 min at 11 (fairly light) on the 6 – 20 Borg RPE scale and 4 min at 15 (hard) on the Borg RPE scale. See Figure 3 for main trial schematic.

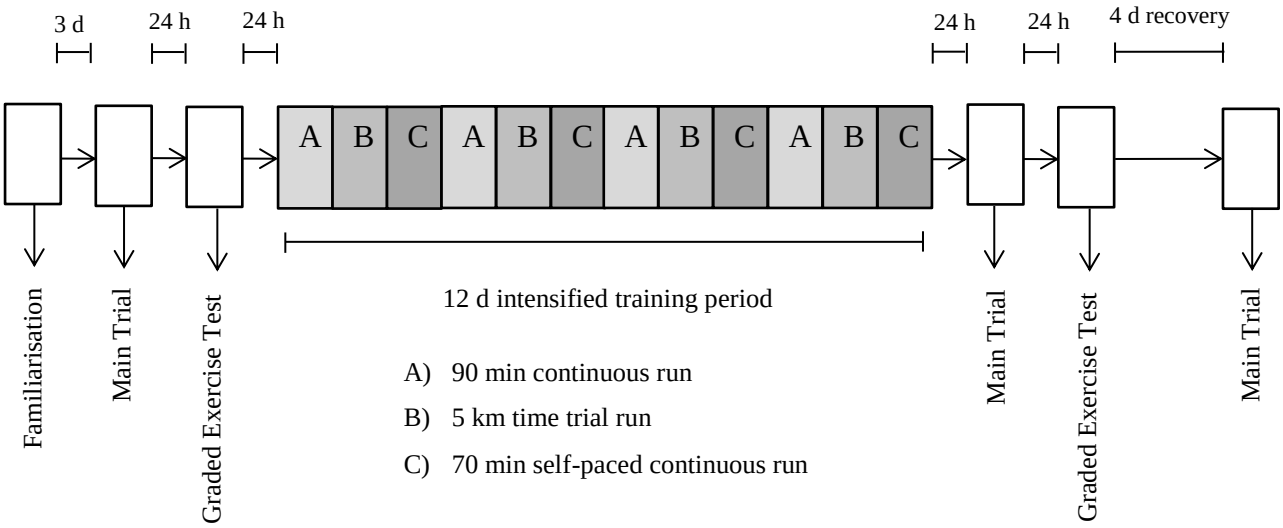

Figure 2 Schematic of overall study.

3.4.1 Familiarisation Trial

Each participant completed a familiarisation trial three days before the main experimental trials began (Figure 2). Each participant was introduced to the equipment and experimental procedures. On arrival, each participant completed a recovery-stress questionnaire for athletes (RESTQ-76 Sport) (appendix e) and Jackson score URS questionnaire (appendix f). The

participant's height (cm) and body mass (kg) were collected pre-exercise, post-exercise and 30 min post-exercise. For the familiarisation trial, participants completed the $RPE_{treadmill}$ bout and a 10 km running TT with a one hour seated rest recovery in between. No saliva samples were collected for this trial.

3.4.2 Main Trial

During the main experimental trials, each participant completed the $RPE_{treadmill}$ bout and a 10 km running TT on a motorised treadmill (Woodway, PPS55 Med-I, Germany) on three separate occasions. The first being 24 h before the maximal exercise test (pre-training), the second being 24 h after the 12 day intensified training period (post-training) and the third being 4 days after the maximal exercise test (post-recovery) (Figure 2). Each participant came to the laboratory at 11:30 to avoid the influence of the circadian rhythm on the hormones. For the main experimental trials, participants were asked to consume a standardised breakfast 4 h before and remained fasted after consumption until the end of each trial. Each participant abstained from alcohol, caffeine and exercise 24 h before the main exercise trials. They were also asked to consume 500 mL of water several hours before each trial to help ensure that they were in a euhydrated state and normal body electrolyte status when they arrived at the laboratory (Sawka *et al.*, 2007). Hydration status was confirmed with a pre-exercise urine osmolality test analysis at the beginning of each main experimental trial.

On arrival, participants completed a RESTQ-76 Sport and Jackson score URS questionnaire. The RESTQ-76 Sport was used to identify the physical and mental stress of the athletes during the past three days and nights prior to exercise and their current capabilities towards recovery (Kellmann and Kallus, 2001). The questionnaire included 48 non-specific and 28 sport-specific items in which each participant answered using a scale ranging from never (0) to always (6). Previous studies have suggested that the RESTQ-76 Sport questionnaire to be a useful tool in highlighting changes in training load in overreached and OTS athletes (Kellmann and Günther, 2000; Nederhof *et al.*, 2008).

The Jackson Score URS questionnaire was used to determine the appearance of whether participants were suffering from a common cold or flu within three days of the main trials (Jackson *et al.*, 1958). Each participant rated the degree of discomfort of their symptoms using the Jackson scoring system: "none at all", "mild", "moderate" and "severe". Symptoms

included sneezing, headache, malaise, nasal discharge, nasal obstruction, sore throat, cough, ear ache, hoarseness, fever, chilliness and joint aches and pains. The use of the Jackson Score URS questionnaire in exercise immunology studies has recently been documented in the Consensus Statement of Immunonutrition and Exercise as a practical and reliable method for reporting URS (Bermon *et al.*, 2017). Previous studies have also shown that this questionnaire to be a good indicator of URS (Hanstock *et al.*, 2016).

Pre-exercise hydration status was determined via a urine sample using an Osmocheck Osmolality Refractometer (Osmocheck, Vitech Scientific, West Sussex, UK). Participants were deemed euhydrated if urine osmolality was <700 mOsmol·kg^{-1} H_2O (Sawka *et al.*, 2007). If the value was exceeded, participants were required to drink more water until they were deemed euhydrated. Two participants failed the osmolality test during the study and were asked to consume more water in order to ensure they were euhydrated before commencement of the study. Both participants passed the osmolality test the second time. Unstimulated saliva samples were collected for three min pre-exercise, post-exercise and 30 min post-exercise for the $RPE_{treadmill}$ bout. The participant's body mass were collected pre-exercise, post-exercise and 30 min post-exercise to determine sweat rate (Sawka *et al.*, 2007). Participants were allowed to consume water *ad libitum* during the $RPE_{treadmill}$ bout and 30 min post-exercise up until the final 10 min before the saliva collection. Water intake was calculated for during exercise ($RPE_{treadmill}$) and post-exercise.

The $RPE_{treadmill}$ bout began at 12:00 and finished at 12:30. Heart rate [HR; (beats·min^{-1}] recorded by telemetry (Polar-FS1, Polar Electro, Kempele, Finland), speed (km·h^{-1}) and RPE using the Borg Scale was collected in the final 30 s of each 1 min and 4 min stage. Following a one hour seated rest recovery, the participant completed a 10 km TT. Time to completion (TTC) and average RPE was recorded at the end of the TT. See Figure 3 for main trial study schematic.

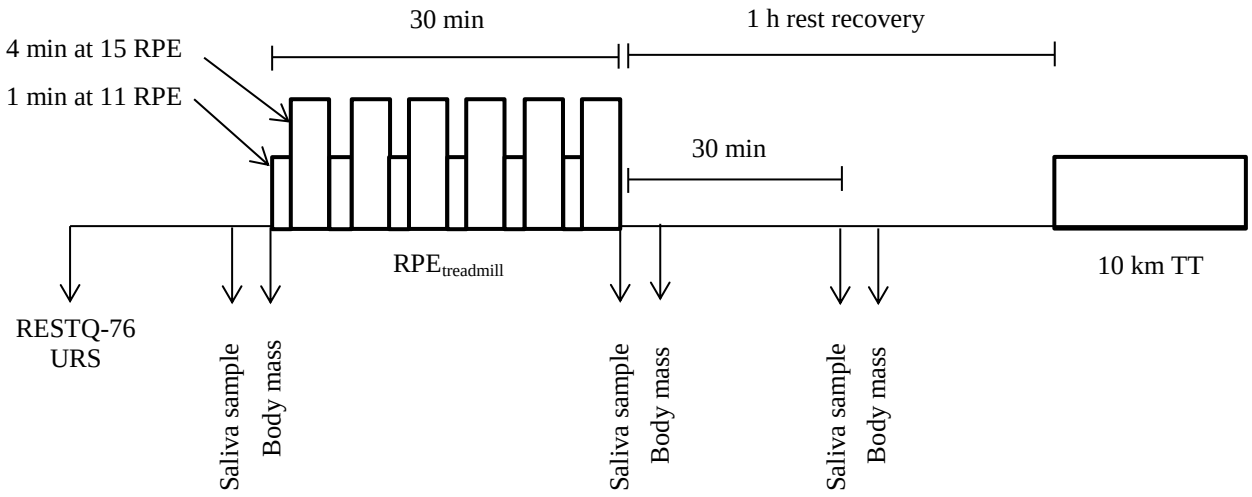

Figure 3 Main trial schematic.

3.5 Graded Exercise Test

Each participant completed a continuous sub-maximal exercise test on the treadmill 24 h after the main exercise trial (Figure 2). This consisted of 4 × 4 min stages (16 min in total). Each participant determined their own starting speed between 6.5 km·h^{-1} and 12 km·h^{-1} and was increased by 1 km·h^{-1} each stage. Throughout the sub-maximal test, a 1% gradient was used as this most accurately reflects the energetic cost of outdoor running (Jones and Doust, 1996).

Thereafter, the participant had a 20 min recovery before completing a maximal graded exercise test to determine the individual's maximal oxygen consumption ($\dot{V}O_{2max}$). The test began with a gradient of 1% and increased by 1% every 1 min until volitional exhaustion. The speed of the $\dot{V}O_{2max}$ test was determined by when the participant reached 150 beats·min^{-1} or 12 RPE during the sub-maximal exercise test. Throughout the sub-maximal and maximal graded exercise test, HR and RPE were recorded at the end of each stage. Expired gas (minute ventilation [$\dot{V}_E$], oxygen consumption [$\dot{V}O_2$], and carbon dioxide production [$\dot{V}CO_2$]) was analysed by an automated breath by breath gas analyser (Metalyser 3B, Cortex, Biophysik, Leipzig, Germany) for the duration of the test. Verbal encouragement was given throughout the test.

3.6 Saliva Collection and Analysis

Each participant was allowed to drink water up to 10 min prior to the saliva collection to ensure that the saliva sample was not diluted as directed by the cortisol and testosterone Enzyme Linked Immunosorbent Assay (ELISA) manufacturers. Throughout the collection period, each participant remained seated and was instructed to swallow the contents of their mouth before collection began. Each participant then provided an unstimulated saliva sample by passive dribble into a 7 mL Bijou tube (Sterilin, Thermo Scientific, Loughborough, UK) with eyes open, head tilted forward and making minimal orofacial movement (as completed in many saliva collection studies e.g. Hough *et al.*, 2011, 2013). A collection time of a minimum of three minutes was allocated in order to allow participants to provide a sufficient quantity of saliva. The time was exceeded if the participants had not provided a sufficient quantity of saliva within the allocated timeframe. Once the saliva collection process was completed, the Bijou tubes were weighed to the nearest milligram to estimate the saliva volume. The saliva density was assumed to be 1.0 $g \cdot mL^{-1}$ (Cole and Eastoe, 1988). The saliva was then transferred into a 1.5 mL Eppendorf tube (Eppendorf, Hamburg, Germany) and was frozen, at –80°C, for later analysis.

3.6.1 Salivary Hormone Analysis

A commercially available ELISA kits (Salimetrics, PA 16803, USA) were used to determine the salivary cortisol and testosterone concentrations according to the manufacturer's instructions. A 96-well microtitre plate were used and pre-coated with monoclonal antibodies to cortisol and rabbit antibodies to testosterone. Absorbance values on the microplate reader (Infinite® 200 PRO, Tecan GmbH, Switzerland) were measured at 450 ± 10 nm. The mean intra-assay coefficients of variation were 4.6% and 4.6% for cortisol and testosterone, respectively. The mean inter-assay coefficients of variation were 6.0% and 9.8% for cortisol and testosterone, respectively.

3.6.2 Salivary Secretory IgA Analysis

All samples were transported to the University of Kent to be analysed over a three day period for SIgA using an in-house ELISA kits. The methodology of the in-house ELISA kit has been

modified from previous research (Leicht, Bishop and Goosey-Tolfrey, 2011) to include a capture antibody specific to the secretory component of human IgA. The use of this in-house ELISA kit has been utilised in previous exercise studies to measure SIgA (Jones *et al.*, 2015; Chidley and Davison, 2017).

SIgA was expressed as absolute concentration and secretion rate. The SIgA secretion rate was calculated using the following equation:

SIgA secretion rate = absolute SIgA concentration × salivary flow rate ($mL \cdot min^{-1}$)

Salivary flow rate = volume of each sample (mL) ÷ collection time of each sample (min)

3.7 Intensified Training Period

Each participant completed a 12 day intensified training period (Figure 2). The training days were completed in the laboratory and on site gym on a motorised treadmill at 1% gradient. The final day of training was completed 24 h before the post-training main exercise trial to ensure that the participants had sufficiently recovered to complete the post-training main exercise trial. To determine the training intensities, velocity at $\dot{V}O_{2max}$ [$v\dot{V}O_{2max}$] was calculated from the sub-maximal exercise test. Each training day consisted of either: (A) 90 min continuous run (70 min at 55% $v\dot{V}O_{2max}$ and 20 min at 75% $v\dot{V}O_{2max}$); (B) 5 km TT; (C) 70 min self-paced continuous run (30 min at 12 RPE, 30 min at 13 RPE and 10 min at 15 RPE). Each participant completed exercise A on day 1, exercise B on day 2 and exercise C on day 3. This three day block of training was repeated four more times (Figure 2). The design of this training period was to reduce the monotony of training. Resting HR, average HR, HR_{max}, average RPE, time and distance measurements were collected after each training session. Temperature (°C) and relative humidity (%) was also collected.

3.7.1 Training Diaries

During the 12 day intensified training period, participants were asked to continue their own training plan and to record each session. This was to ensure that participants were increasing their training load to induce a state of overreaching. Each participant was provided with a training log (appendix g), a Borg scale and a HR monitor. Participants were asked to wear the

HR monitor and record the duration, resting HR, average HR, HR_{max} and average RPE for each additional exercise session that was completed outside the laboratory. Each participant also provided a 12 day training programme for a normal period of training to act as a control measure and this included the same measured variables.

Training load was determined using Banister training impulse (TRIMP). TRIMP was calculated using duration of the training session, resting HR, average HR and HR_{max} during the exercise sessions (see equation below).

$$\text{TRIMP} = \text{duration (min)} \times \Delta\text{HR ratio} \times Y$$

$$\Delta\text{HR ratio} = \frac{HR_{average} - HR_{rest}}{HR_{max} - HR_{rest}}$$

Where $Y = 0.64e^{1.92x}$, $e = 2.712$ and $x = \Delta$HR ratio.

3.8 Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics Version 22 (SPSS Inc., Chicago, IL). Descriptive data was presented as a mean ± standard deviation (*SD*) and percentile differences. Quantile-quantile (Q-Q) plots and Shapiro-Wilk test were used to check the normal distribution of data. In the case of non-normally distributed data, logarithmic transformations were performed on the data. If normality was violated again following logarithmic transformations, a Friedman or multiple Wilcoxon signed ranked non-parametric test were completed on the original data. In the case of normal distribution, data were analysed using a two-way (trial × time) repeated-measures analysis of variance (ANOVA). A Student's paired samples *t*-tests and Bonferroni *post hoc* test was used to locate the differences in the event of a significant interaction effect. Further analysis was completed on participants who completed all three main trials ($n = 4$) and individual analysis was also completed on them. Cohen's *d* ES were calculated by dividing the difference between the mean of two groups by the pooled SD (Cohen, 1988, 1992). As per Cohen's guidelines, an ES equal to or greater than 0.2, 0.5 and 0.8 represent a small, moderate and large effect. The two-tailed alpha level of significance testing was set as $p < 0.05$.

CHAPTER 4 Results

For the present study, four participants completed 17 visits (all visits except the post-recovery main trial) and four participants completed 18 visits in total. Each participant attended a familiarisation trial before the main experimental trials began (visit 1). Three main trials (visit 2, 16 and 18) and two graded exercise tests (visit 3 and 17) were completed by each participant. Participants also completed a 12 day intensified training period (visit 4-15). See Figure 4.

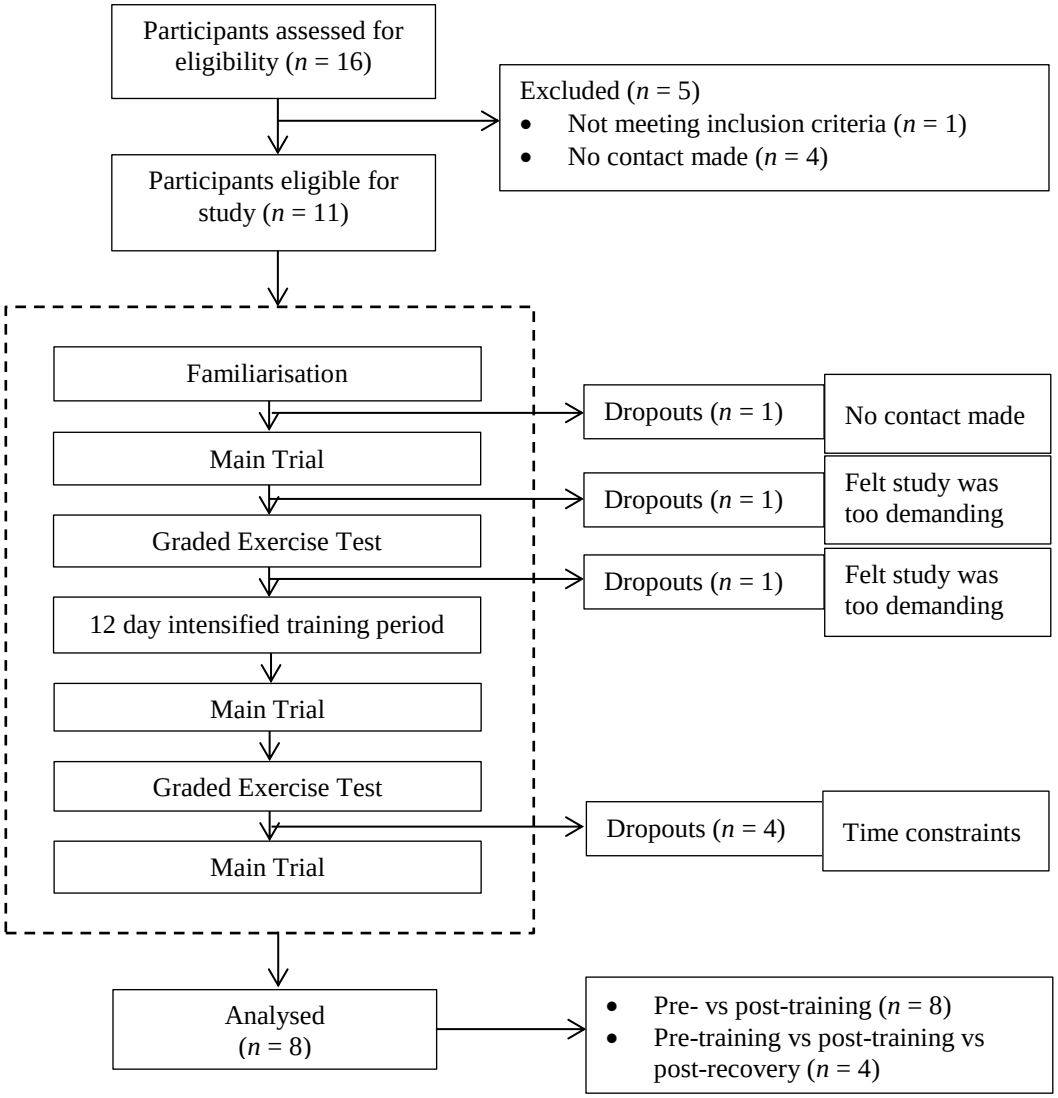

Figure 4 CONSORT diagram.

4.1 Tests of Normality

The assumption of normality was evaluated as a whole group between pre- and post-training (n = 8) and determined to be satisfied in training load, $\dot{V}O_{2max}$, hydration status, 10 km TT performance, HR, speed and salivary testosterone. The assumption of normality was violated in RPE, salivary cortisol, SIgA concentration, SIgA secretion rate, RESTQ-76 Sport and URS questionnaires. Logarithmic transformations were performed on these variables and were re-evaluated for normality. Salivary cortisol, SIgA concentration and secretion rate were deemed to be satisfied and RPE, REST-76 Sport and URS questionnaires were violated following re-evaluation.

The assumption of normality was also evaluated on participants who completed the pre-training, post-training and post-recovery main trials (n = 4) and determined to be satisfied in hydration status, 10 km TT performance, speed and salivary testosterone. The assumption of normality was violated in HR, RPE, salivary cortisol, SIgA concentration, SIgA secretion rate, RESTQ-76 Sport and URS questionnaires. Logarithmic transformations were performed on these variables and were re-evaluated for normality. SIgA concentration was deemed to be satisfied and HR, RPE, SIgA secretion rate, RESTQ-76 Sport and URS questionnaires were violated following re-evaluation.

4.2 Training Load

All participants completed the 12 day intensified training period. During the 12 day intensified training period, the participants completed 16.7 ± 6.3 h (average of 58.0 ± 9.0 min per day) of running compared to the normal 12 day training period in which participants completed 8.0 ± 7.8 h (average of 44 ± 31 min per day). The overall TRIMP score significantly increased by 118% from the normal 12 day training period (663 ± 616) to the 12 day intensified training period (1447 ± 471) (p < 0.001). A large ES was observed (1.12).

4.3 Maximum Oxygen Consumption

Pre- (59 ± 6 $mL \cdot kg^{-1} \cdot min^{-1}$) and post-training (59 ± 7 $mL \cdot kg^{-1} \cdot min^{-1}$) $\dot{V}O_{2max}$ did not significantly differ (p = 0.911). This placed them above 90% of men between 20 and 29 years old on the aerobic capacity spectrum according to the American College of Sports Medicine

(American College of Sports Medicine, 2018). The participant's level of training ranged from three to seven days per week with levels of performance ranging from recreational to competitive.

4.4 Hydration Status

No significant difference was found between pre- (278 ± 245 mOsmol·kg^{-1}) and post-training (439 ± 284 mOsmol·kg^{-1}) urine osmolality ($p > 0.05$). No significant main effect ($F_{1, 3} = 0.492$, $p = 0.534$) was found between pre-training (120 ± 114 mOsmol·kg^{-1}), post-training (380 ± 314 mOsmol·kg^{-1}) and post-recovery (85 ± 30 mOsmol·kg^{-1}).

4.5 10 km Time Trial Performance

No significant difference was found between pre- and post-training 10 km TT performances ($p = 0.575$) (Figure 5). Although no significant differences were found, average 10 km TT performance were lower (3%) pre-training (45.2 ± 5.3 min) compared to post-training (46.4 ± 4.7 min). Two participants were removed from the data analysis due to failing to start the post-training 10 km TT. A small ES between pre- and post-training (0.24) was observed.

No significant main effect ($F_{2, 4} = 3.757$, $p = 0.121$) was found between pre-training (42.3 ± 5.7 min), post-training (46.9 ± 9 min) and post-recovery (40.8 ± 6.5 min) (Figure 6). The calculated ES for 10 km TT performance showed a small ES between pre-training and post-recovery (0.26), a moderate ES between pre- and post-training (0.76) and a large ES between post-training and post-recovery (0.89).

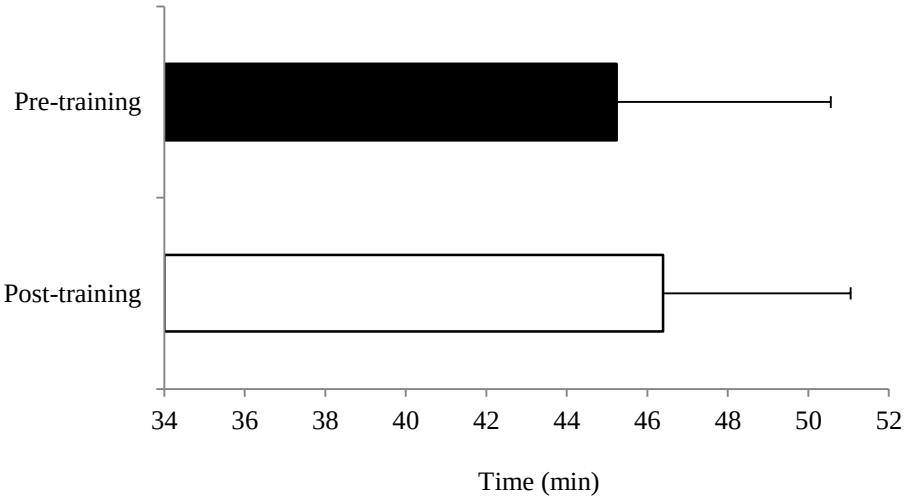

Figure 5 Pre- and post-training average 10 km time trial performance (min) (n = 6). Values are mean ± *SD*.

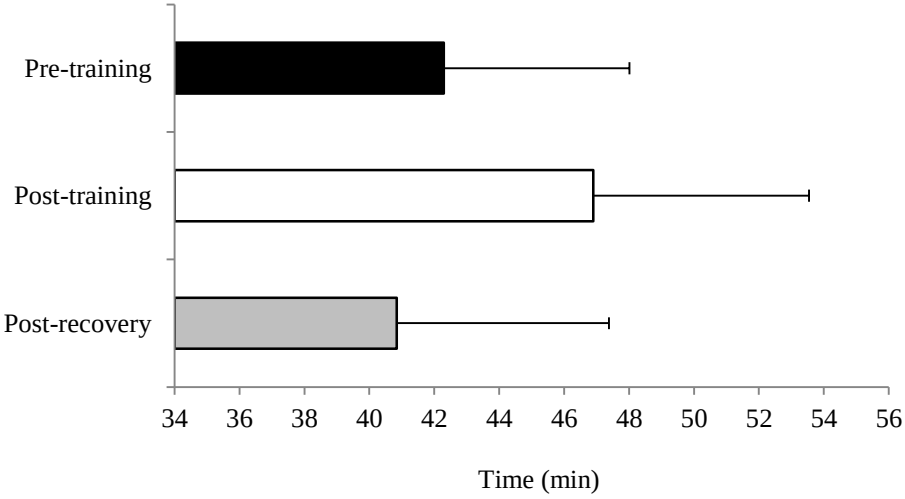

Figure 6 Pre-training, post-training and post-recovery average 10 km time trial performance (min) (n = 3). Values are mean ± *SD*.

4.6 Physiological Responses

No significant differences in HR, speed and RPE ($p > 0.05$) responses to the $RPE_{treadmill}$ bout were found between pre- (HR: 150 ± 4 beats·min^{-1}; speed: 10.8 ± 0.5 km·h^{-1}; RPE: 13 ± 0) and post-training (HR: 146 ± 3 beats·min^{-1}; speed: 11.1 ± 0.4 km·h^{-1}; RPE: 13 ± 0) (Table 6).

No significant differences in HR, speed and RPE ($p > 0.05$) responses to the the $RPE_{treadmill}$ bout were found between pre-training (HR: 148 ± 19 beats·min^{-1}; speed: 10.6 ± 3.8 km·h^{-1}; RPE: 13 ± 0), post-training (HR: 142 ± 19 beats·min^{-1}; speed: 10.6 ± 3.1 km·h^{-1}; RPE: 13 ±

0) and post-recovery (HR: 147 ± 5 beats·min^{-1}; speed: 11.1 ± 2.8 km·h^{-1}; RPE: 13 ± 0) (Table 7).

Table 6 Pre- and post-training average HR (beats·min^{-1}), speed (km·h^{-1}) and RPE responses to the $RPE_{treadmill}$ bout (n = 8).

Trial	HR (beats·min^{-1})	Speed (km·h^{-1})	RPE
Pre-training	150 ± 4	10.8 ± 0.5	13 ± 0
Post-training	146 ± 3	11.1 ± 0.4	13 ± 0

Values are mean ± *SD*.

Table 7 Pre-training, post-training and post-recovery average HR (beats·min^{-1}), speed (km·h^{-1}) and RPE responses to the $RPE_{treadmill}$ bout (n = 4).

Trial	HR (beats·min^{-1})	Speed (km·h^{-1})	RPE
Pre-training	148 ± 19	10.6 ± 3.8	13 ± 0
Post-training	142 ± 19	10.6 ± 3.1	13 ± 0
Post-recovery	147 ± 5	11.1 ± 2.8	13 ± 0

Values are mean ± *SD*.

4.7 Salivary Hormone Responses

4.7.1 Salivary Testosterone

No significant main effect for trial ($F_{1, 7}$ = 1.222, p = 0.737) and trial × time interaction ($F_{2, 14}$ = 0.427, p = 0.661) were found between pre- and post-training. However, a significant main effect for time ($F_{2, 14}$ = 4.833, p = 0.025) was found with a t-test analysis indicating a significant increase (36%) from pre- (484.0 ± 194.3 pmol·L^{-1}) to post-exercise (658.2 ± 309.6 pmol·L^{-1}) saliva samples pre-training only (p = 0.02) (Figure 7). The exercise-induced salivary testosterone response (pre- to post-exercise) decreased by 42% from pre- to post-training. A small ES between pre- and post-training (0.43) was observed.

No significant main effect for trial ($F_{2, 6}$ = 2.224, p = 0.189) and trial × time interaction ($F_{2.105, 6.316}$ = 3.43, p = 0.097) were found between pre-training, post-training and post-recovery. A

significant main effect of time ($F_{2, 6}$ = 9.308, p = 0.014) was found with a *t*-test analysis indicating a significant increase (101%) from pre- (424 ± 113 pmol·L^{-1}) to post-exercise (851 ± 307 pmol·L^{-1}) saliva samples post-recovery only (p = 0.027) (Figure 8). The calculated ES for the exercise-induced salivary testosterone response showed a moderate ES between pre- and post-training (0.67) and a large ES between pre-training and post-recovery (1.07) and post-training and post-recovery (1.24).

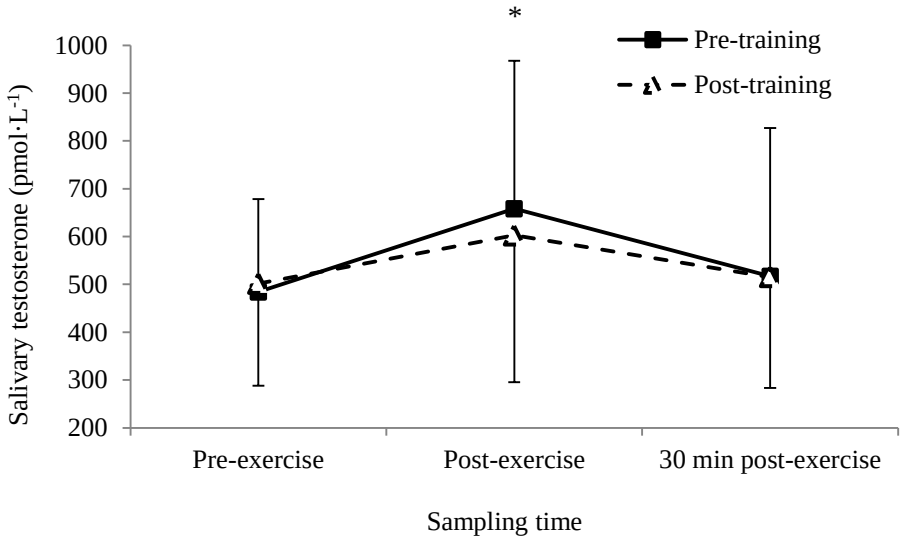

Figure 7 Pre- and post-training average salivary testosterone responses (pmol·L^{-1}) to the $RPE_{treadmill}$ bout (n = 8). Values are mean ± *SD*.

*denotes a significant increase from pre-exercise saliva sample at pre-training (p < 0.05).

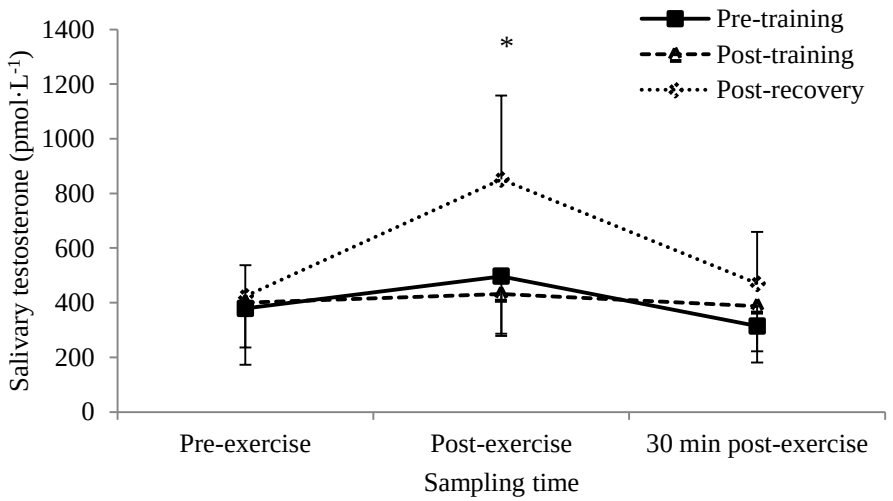

Figure 8 Pre-training, post-training and post-recovery average salivary testosterone responses (pmol·L^{-1}) to the $RPE_{treadmill}$ bout (n = 4). Values are mean ± *SD*.

*denotes a significant increase from pre-exercise saliva sample at post-recovery (p < 0.05).

4.7.2 Salivary Cortisol

No significant main effect for trial ($F_{1, 7}$ = 0.009, p = 0.928) and time ($F_{2, 14}$ = 2.313, p = 0.136) were found between pre- and post-training (Figure 9). The exercise-induced salivary cortisol response (pre- to post-exercise) increased by 675% from pre- to post-training. A small ES between pre- and post-training (0.49) were observed.

No significant main effect for trial ($F_{2, 6}$ = 2.487, p = 0.163) and time ($F_{2, 6}$ = 1.496, p = 0.297) were found between pre-training, post-training and post-recovery (Figure 10). The calculated ES for exercise-induced salivary cortisol response showed a small ES between pre- and post-training (0.4) and post-training and post-recovery (0.33) and a large ES between pre-training and post-recovery (0.92).

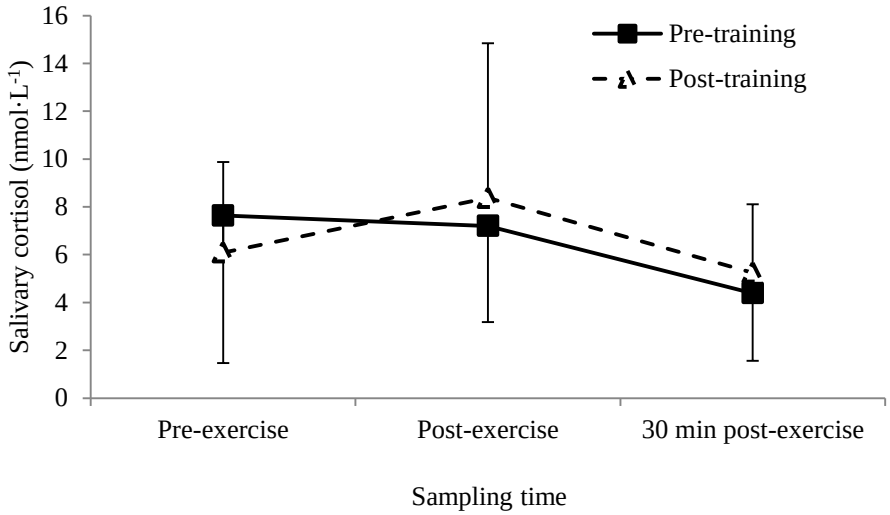

Figure 9 Pre- and post-training average salivary cortisol responses (nmol·L^{-1}) to the $RPE_{treadmill}$ bout (n = 8). Values are mean ± *SD*.

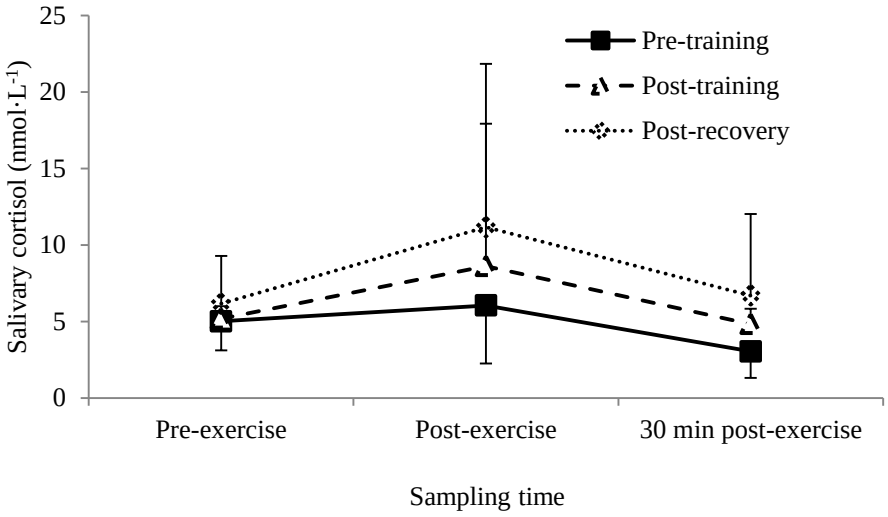

Figure 10 Pre-training, post-training and post-recovery average salivary cortisol responses (nmol·L^{-1}) to the $RPE_{treadmill}$ bout (n = 4). Values are mean ± *SD*.

4.8 Salivary Secretory IgA Responses

4.8.1 Salivary Secretory IgA Concentrations

The resting SIgA concentrations did not significantly differ (p = 0.504) between pre- (132.5 ± 61.8 mg·L^{-1}) and post-training (125.7 ± 73.6 mg·L^{-1}) (Figure 11). A significant main effect for trial ($F_{2, 6}$ = 5.381, p = 0.046) was found between pre-training, post-training and post-recovery with a *t*-test analysis indicating a significant decrease (p =0.028) from pre- (117.7 ± 17.9 mg·L^{-1}) to post-training only (91.3 ± 12.5 mg·L^{-1}) (Figure 12).

No significant main effect for trial ($F_{1, 7}$ = 0.001, p = 0.978) and time ($F_{1.144, 8.007}$ = 0.973, p = 0.366) were found between pre- and post-training (Figure 11). The exercise-induced SIgA concentration (pre- to post-exercise) decreased by 4% from pre- to post-training. No ES was observed between pre- and post-training.

No significant main effect for trial ($F_{1, 7}$ = 0.002, p = 0.969) and time ($F_{2, 14}$ = 1.262, p = 0.313) were found between pre-training, post-training and post-recovery (Figure 12). The calculated ES for the exercise-induced SIgA concentration showed a small ES between pre- and post-training (0.43), a moderate ES between pre-training and post-recovery (0.74) and a large ES between post-training and post-recovery (1.23).

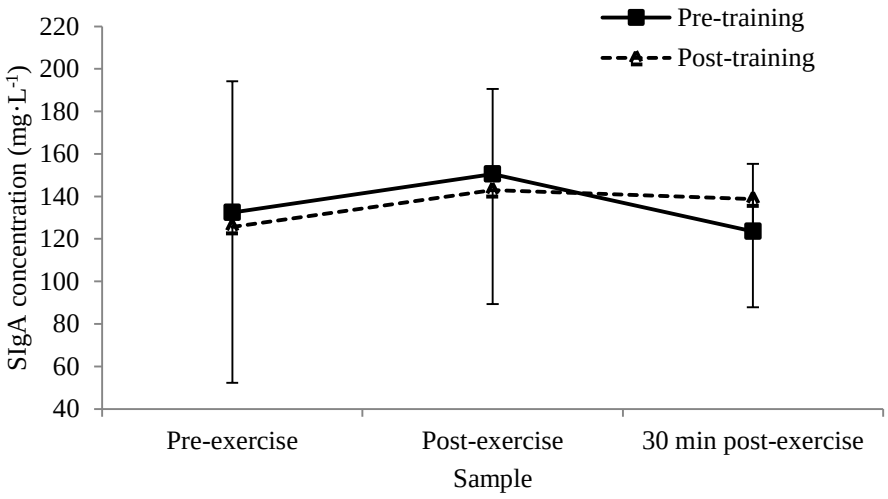

Figure 11 Pre- and post-training average SIgA concentrations (mg·L^{-1}) to the $RPE_{treadmill}$ bout (n = 8). Values are mean ± *SD*.

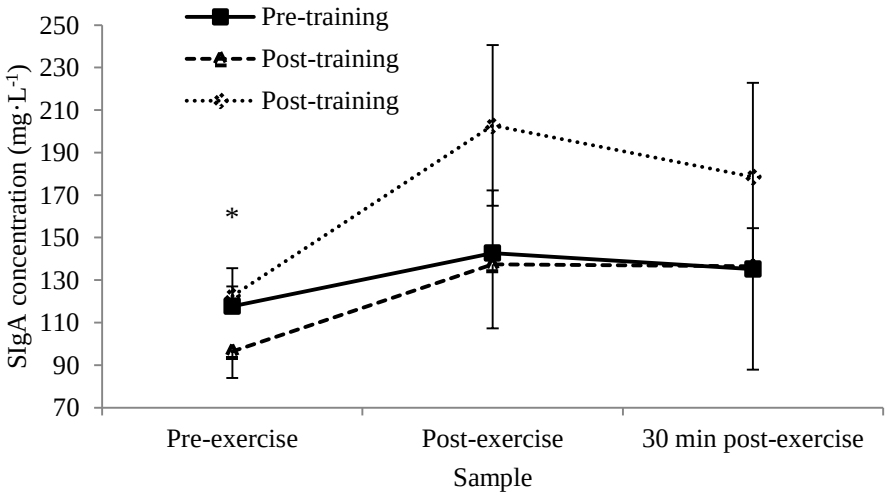

Figure 12 Pre-training, post-training and post-recovery average SIgA concentrations (mg·L^{-1}) to the $RPE_{treadmill}$ bout (n = 4). Values are mean ± *SD*.

*denotes a significant decrease for pre-exercise saliva sample from pre- to post-training ($p < 0.05$).

4.8.2 Salivary Secretory IgA Secretion Rate

The resting SIgA secretion rate did not significantly differ (p = 0.721) from pre- (39 ± 30 µg·min^{-1}) to post-training (43 ± 18 µg·min^{-1}) (Figure 13). No significant main effect for trial ($F_{2, 6}$ = 0.175, p = 0.843) was found between pre-training (46.6 ± 28.4 µg·min^{-1}), post-training (40.0 ± 13.1 µg·min^{-1}) and post-recovery (45.9 ± 29.2 µg·min^{-1}) (Figure 14).

No significant main effect for trial ($F_{1, 7}$ = 0.002, p = 0.969) and time ($F_{2, 14}$ = 1.262, p = 0.313) were found between pre- and post-training (Figure 13). The exercise-induced SIgA secretion rate (pre- to post-exercise) decreased by 309% from pre- to post-training. A small ES between pre- and post-training (0.32) was observed.

No significant difference (p = 0.058) was found between pre-training, post-training and post-recovery (Figure 14). The calculated ES for the exercise-induced SIgA secretion rate showed a small ES between pre- and post-training (0.4) and a large ES between post-training and post-recovery (1.61) and pre-training and post-recovery (1.18).

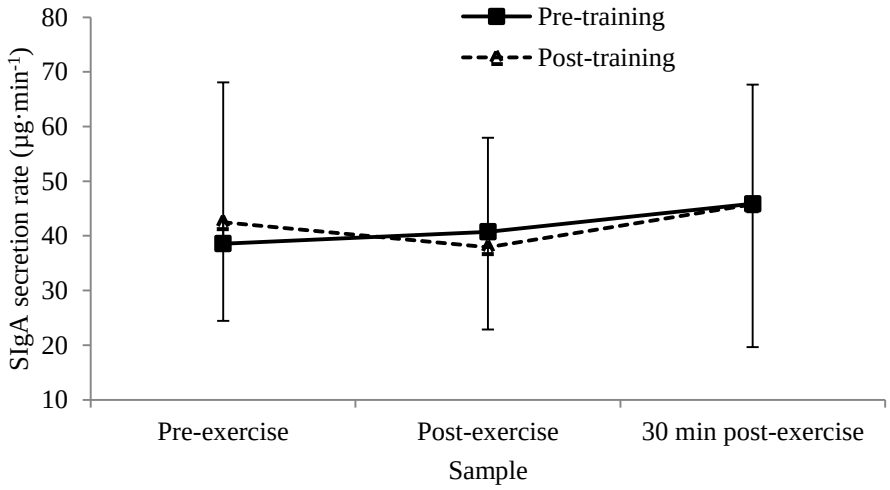

Figure 13 Pre- and post-training average SIgA secretion rate (µg·min^{-1}) to the $RPE_{treadmill}$ bout (n = 8). Values are mean ± *SD*.

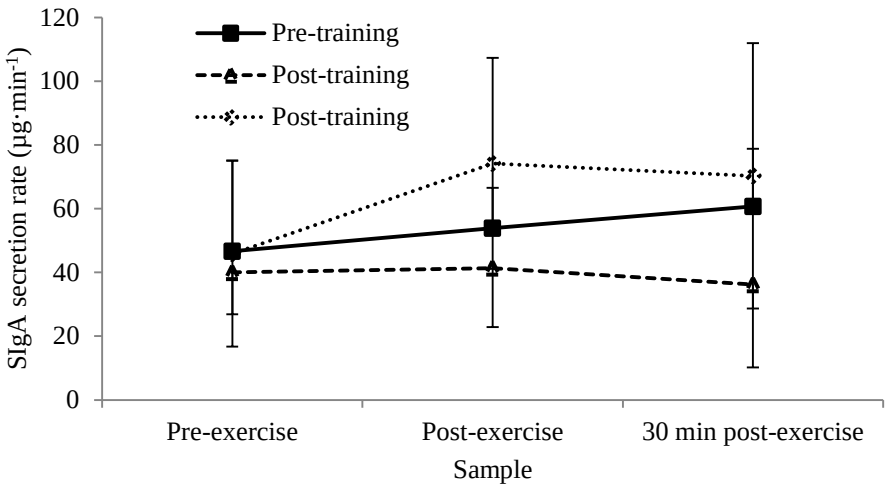

Figure 14 Pre-training, post-training and post-recovery average SIgA secretion rate (µg·min^{-1}) to the $RPE_{treadmill}$ bout (n = 4). Values are mean ± *SD*.

4.9 RESTQ-76 Sport Questionnaire

Analysis of the RESTQ-76 Sport questionnaires revealed that general stress, general recovery, sport stress and sport recovery category scores did not significantly differ from pre- to post-training (p > 0.05) (Figure 15). Analysis of individual statements revealed no significant differences occurred between the main trials (p > 0.05) other than social relaxation was significantly higher post-training compared to pre-training (p = 0.03). A small ES for general stress (0.29), sport stress (0.41) and sport recovery (0.29) and a moderate ES for general recovery (0.55) between pre- and post-training were observed.

A significant difference in sport stress ($F_{2, 6}$ = 6.552, p = 0.031) was found with a t-test indicating sport stress decreased from post-training to post-recovery (p = 0.041) (Figure 16). No significant differences in general stress ($F_{2, 6}$ = 4.067, p = 0.077), general recovery ($F_{2, 6}$ = 1.131, p = 0.383) and sport recovery ($F_{2, 6}$ = 0.41, p = 0.365) occurred between pre-training, post-training and post-recovery. A large ES for general stress (0.90), general recovery (1.07) and sport stress (0.95) between pre- and post-training were observed. A moderate ES for general recovery (0.78) and sport stress (0.77) and a large ES for sport recovery (0.82) between pre-training and post-recovery were observed.

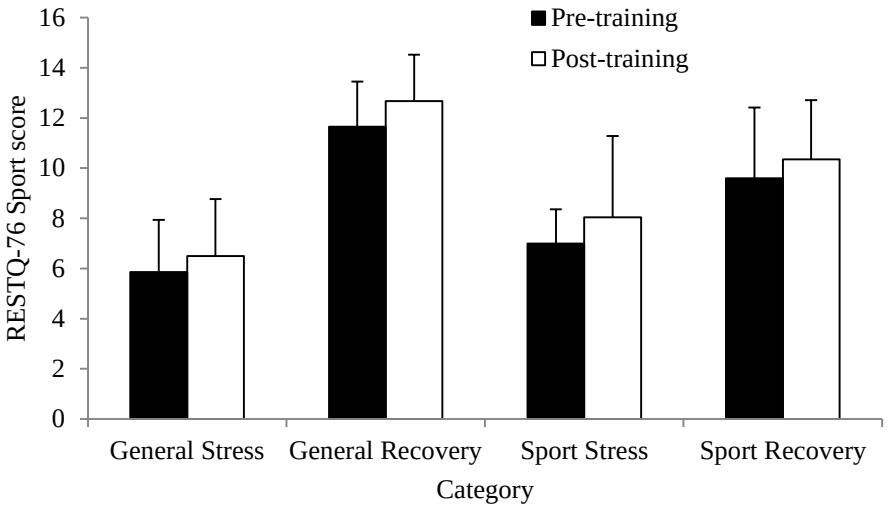

Figure 15 Pre- and post-training RESTQ-76 Sport general stress, general recovery, sport stress and sport recovery scores (n = 8). Values are mean ± *SD*.

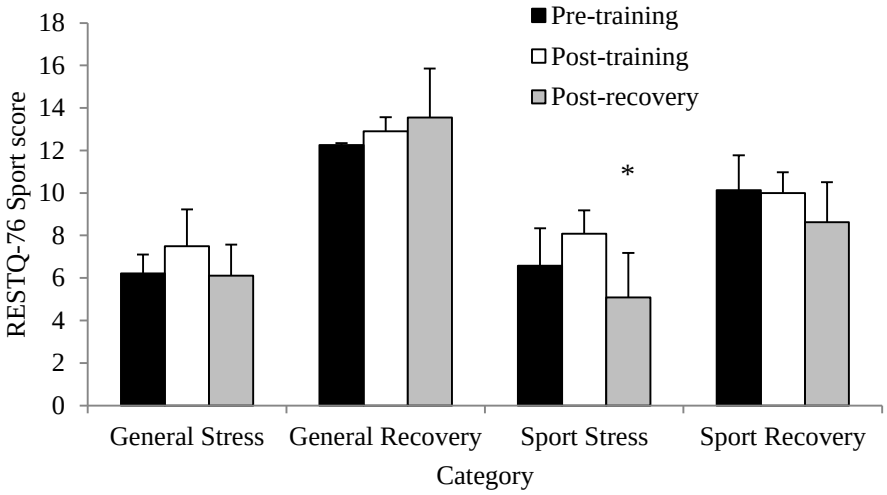

Figure 16 Pre-training, post-training and post-training RESTQ-76 Sport general stress, general recovery, sport stress and sport recovery scores (n = 4). Values are mean ± *SD*.
*denotes a significant decrease from post-training to post-recovery ($p < 0.05$).

4.10 Upper Respiratory Symptoms

No significant differences between pre- and post-training average URS scores were observed ($p > 0.05$), with the exception of headaches, which were significantly increased from pre- to post-training ($p = 0.046$) (Figure 17). A moderate ES between pre- and post-training (0.75) was observed.

No significant differences were observed for symptoms associated with URS between pre-training, post-training and post-recovery ($p > 0.05$) (Figure 18). The calculated ES for URS found a small ES between post-training and post-recovery (0.44) and a moderate ES between pre- and post-training (0.51). No ES between pre-training and post-recovery was observed.

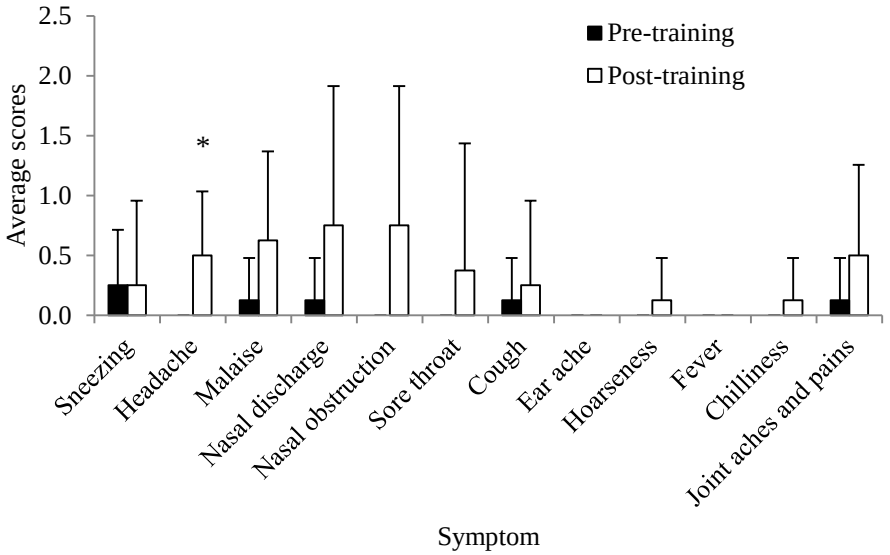

Figure 17 Average pre- and post-training upper respiratory symptom (URS) scores ($n = 8$). Values are mean ± *SD*.

*denotes a significant increase from pre-training ($p < 0.05$).

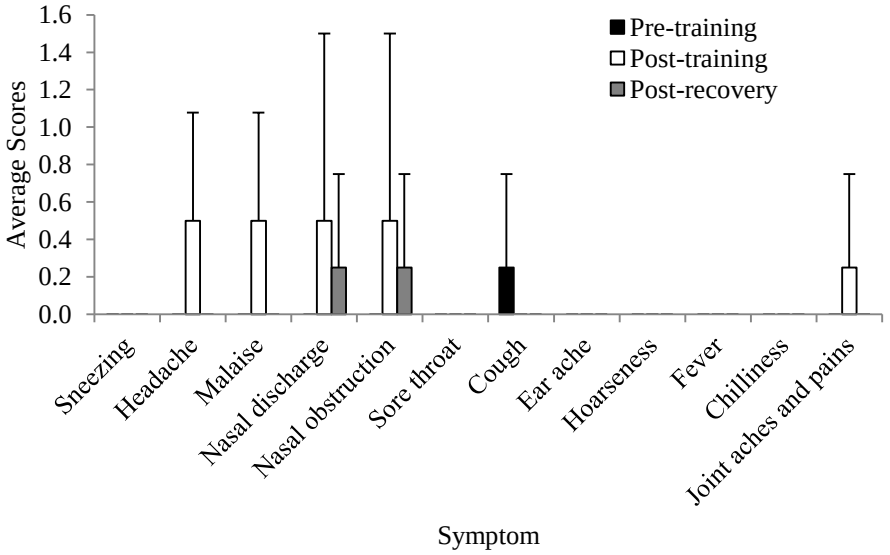

Figure 18 Average pre-training, post-training and post-recovery upper respiratory symptom (URS) scores ($n = 4$). Values are mean ± *SD*.

4.11 Individual Responses

From all the participants that completed the study (n = 8), only four participants completed the post-recovery main trial. From these four participants, two participants (participants 2 and 7) have been highlighted as FOR due to showing signs associated with this state (i.e. a decrease in 10 km TT performance from pre- to post-training and a *super-compensatory* performance improvement post-recovery which is primary indicator of FOR). No participants showed signs of NFOR or OTS.

4.11.1 10 km Time Trial Performance

Participant 2, 3 and 7 pre-training 10 km TT performance were 45.0 min, 46.2 min and 35.7 min. Compared to pre-training, participant 2, 3 and 7 post-training 10 km TT performance were 18%, 3% and 12% slower (participant 2: 53.2 min; participant 3: 47.5 min; participant 7: 40.0 min). Participant 2 and 7 post-recovery 10 km TT performance were 8% and 5% quicker compared to pre-training and 22% and 15% quicker compared to post-training (participant 2: 41.5 min; participant 7: 34.0 min). Participant 3 post-recovery 10 km TT performances were 2% slower compared to pre-training and 1% quicker compared to post-training. Individual 10 km TT performances are presented in Figure 19.

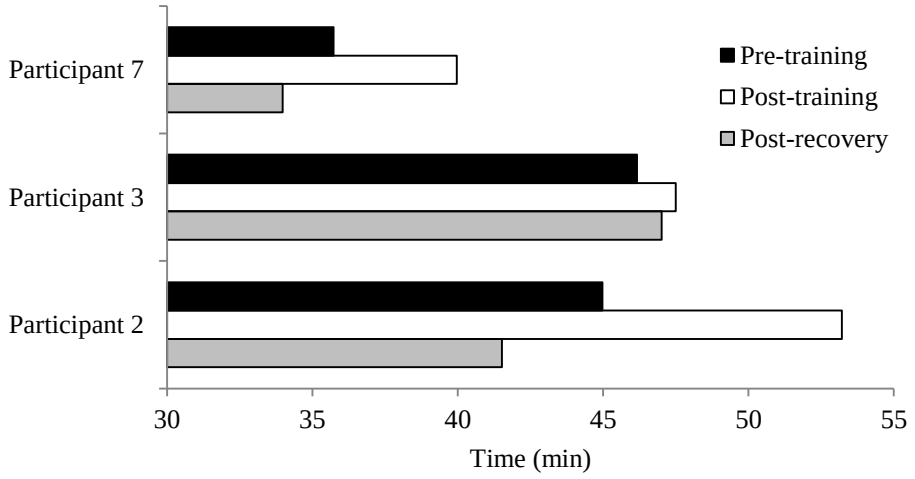

Figure 19 Individual 10 km time trial performances (min) pre-training, post-training and post-recovery.

4.11.2 Salivary Hormone Responses

Pre-training exercise-induced salivary testosterone response (pre- to post-exercise) decreased by 17% in participant 1 and increased by 67%, 41% and 49% in participant 2, 3 and 7. Compared to pre-training, post-training exercise-induced response increased by 268% in participant 1, decreased by 93% and 144% in participant 2 and 7 and did not change in participant 3. Individual exercise-induced salivary testosterone responses are presented in Figure 20.

Pre-training exercise-induced salivary cortisol response (pre- to post-exercise) increased by 12%, 50% and 82% in participant 1, 2 and 3 and decreased by 30% in participant 7. Compared to pre-training, post-training exercise-induced response increased by 50% and 244% in participant 1 and 3 and decreased by 100% and 20% in participant 3 and 7. Individual exercise-induced salivary cortisol responses are presented in Figure 21.

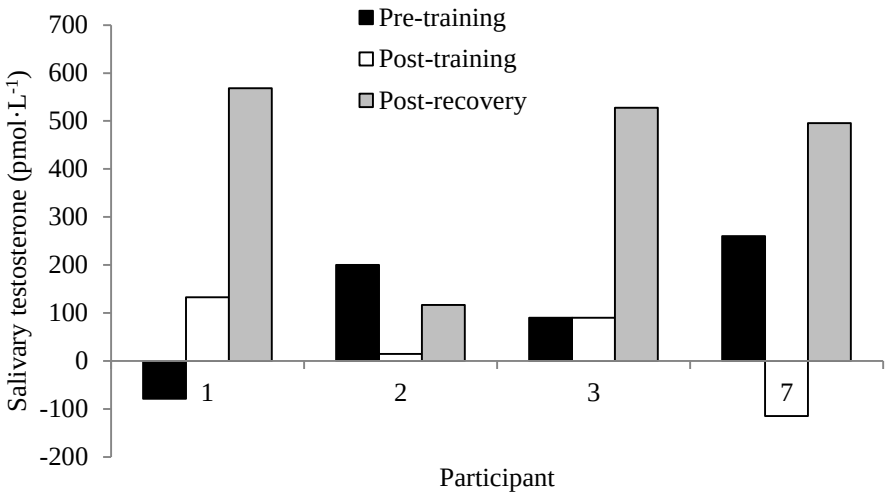

Figure 20 Absolute change of the individual exercise-induced salivary testosterone responses ($pmol \cdot L^{-1}$) to the $RPE_{treadmill}$ bout pre-training, post-training and post-recovery.

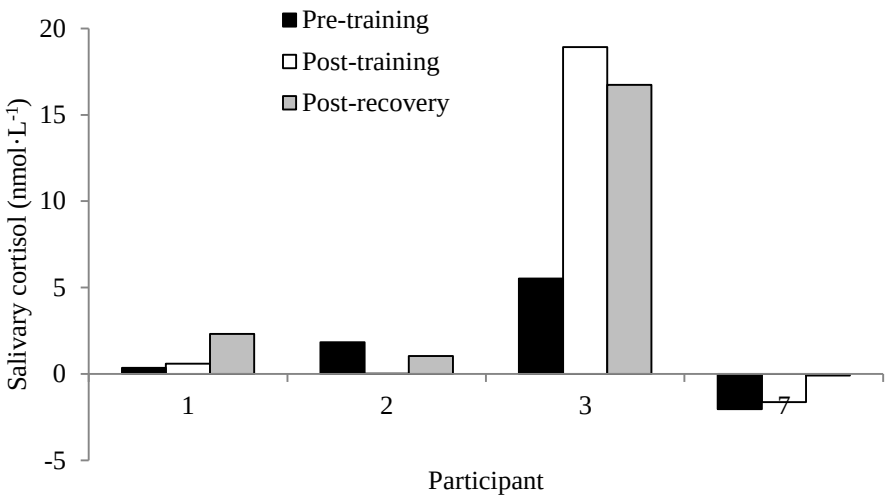

Figure 21 Absolute change of the individual exercise-induced salivary cortisol responses ($nmol \cdot L^{-1}$) to the $RPE_{treadmill}$ bout pre-training, post-training and post-recovery.

4.11.3 Salivary Secretory IgA Responses

Pre-training exercise-induced SIgA concentrations increased by 45% and 66% in participant 1 and 2 and decreased by 13% and 1% in participant 3 and 7. Compared to pre-training, post-training exercise-induced response increased by 67%, 33% and 60% in participant 1, 3 and 7 and decreased by 36% participant 2. Individual exercise-induced SIgA concentrations are presented in Figure 22.

Pre-training exercise-induced SIgA secretion rate increased by 163% and 70% in participant 1 and 7 and decreased by 1% and 24% in participant 2 and 3. Compared to pre-training, post-training exercise-induced secretion rate decreased by 140% and 114% in participant 1 and 7 and increased by 1643% and 146% in participant 2 and 3. Individual exercise-induced SIgA secretion rate are presented in Figure 23.

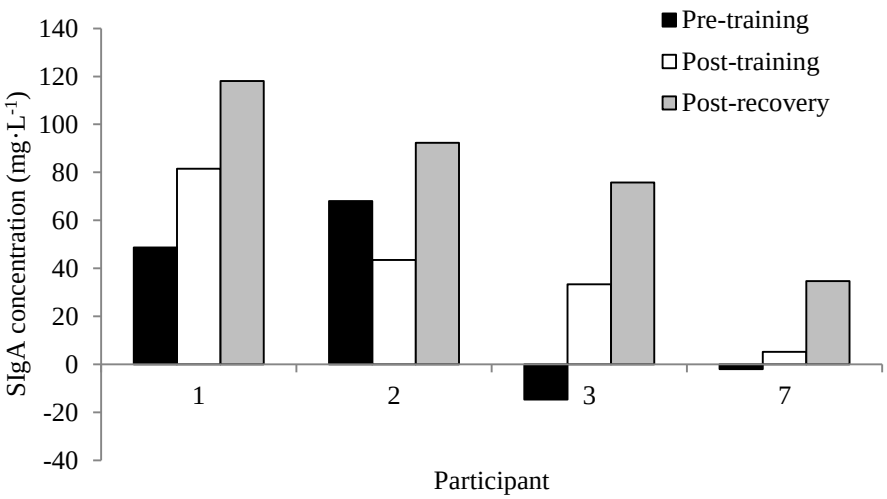

Figure 22 Absolute change of the individual exercise-induced SIgA concentrations (mg·L^{-1}) to the $RPE_{treadmill}$ bout pre-training, post-training and post-recovery.

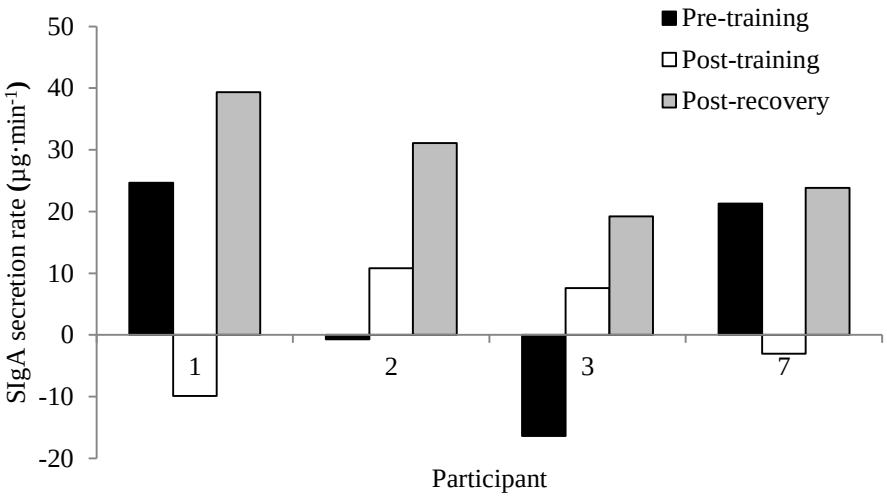

Figure 23 Absolute change of the individual exercise-induced SIgA secretion rate (µg·min^{-1}) to the $RPE_{treadmill}$ bout pre-training, post-training and post-recovery.

4.11.4 RESTQ-76 Sport Questionnaire

Participant 1 sport stress scores were 3% lower pre- to post-training and 8% lower post-training to post-recovery. Participant 2, 3 and 7 sport stress scores were 86%, 9% and 23% higher from pre- (participant 2: 5.0; participant 3: 6.7; participant 7: 5.7) to post-training (participant 2: 9.3; participant 3: 7.3; participant 7: 7.0). Participant 2, 3 and 7 post-recovery

sport stress scores (participant 2: 4.7; participant 3: 4.7; participant 7: 3.0) were 50%, 36% and 57% lower compared to post-training and 6%, 30% and 47% lower compared to pre-training. Individual sport stress scores are presented in Figure 24.

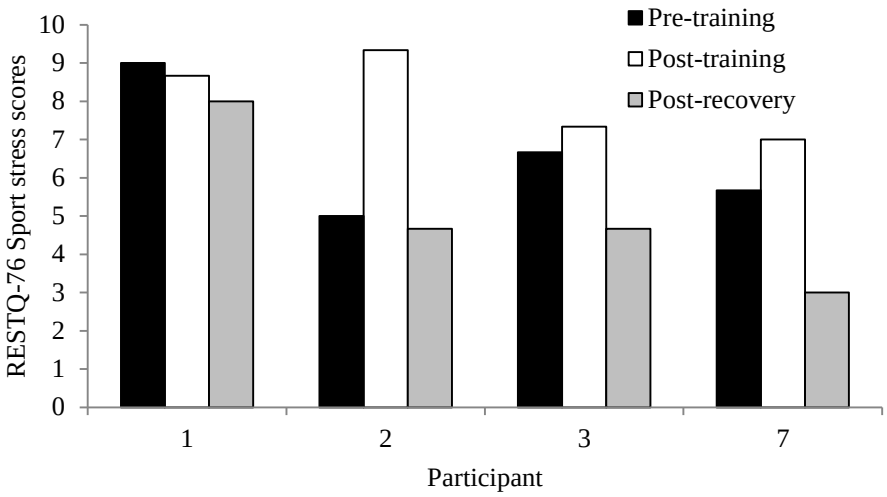

Figure 24 Individual RESTQ-76 Sport stress scores.

4.11.5 Upper Respiratory Symptoms

Participant 1 reported mild symptoms of coughing pre-training and mild symptoms of headache, malaise and joint aches and pains post-training. No URS were reported post-recovery. Participant 2 and 3 reported no URS pre-training, post-training and post-recovery. Participant 7 reported no URS pre-training but did report mild symptoms of headache and malaise and moderate symptoms of nasal discharge and obstruction post-training. Participant 7 also reported mild symptoms of nasal discharge and obstruction post-recovery. Individual URS reported are presented in Figure 25.

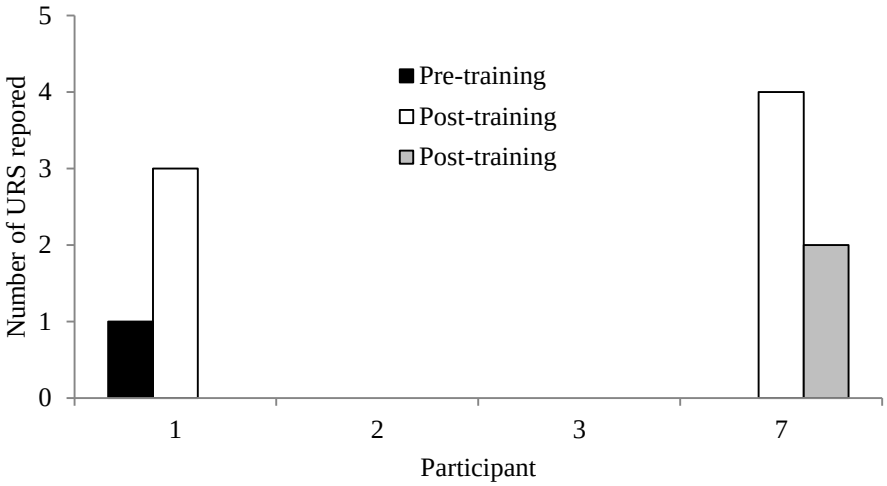

Figure 25 Individual upper respiratory symptoms (URS) reported pre-training, post-training and post-recovery.

CHAPTER 5 Discussion

5.1 Aims of Study

The present study aimed to determine if there was any hormonal alteration of cortisol and testosterone and mucosal immunological responses of SIgA to a 30 min high-intensity running bout ($RPE_{treadmill}$) before and after a 12 day intensified training period. More specifically, it aimed to establish a reliable biological marker and useful tool that may aid in the identification of overreaching (specifically FOR) and therefore the prevention of the OTS occurring in athletes.

5.2 Key Findings

The primary finding of the present study was that the exercise-induced salivary testosterone response significantly increased pre-training with no exercise-induced response found post-training. The exercise-induced salivary cortisol response did not significantly differ from pre- to post-training. No significant difference in the 10 km TT performance was found however, a small decrease was observed from pre- to post-training (ES = 0.24). Moreover, SIgA concentrations and secretion rate did not significantly differ between pre- and post-training. Although, the calculated ES showed that a small decrease in secretion rate did occur post-training (ES = 0.32). Analysis of the four participants who completed all three main trials showed that the exercise-induced salivary testosterone, cortisol and SIgA concentrations and secretion rates did not significantly differ between pre-training, post-training and post-recovery. However, the calculated ES for salivary testosterone showed that compared to pre-training, a moderate decrease occurred post-training (ES = 0.67) and a large increase occurred post-recovery (ES = 1.07). Individual analysis of all four participants that completed all main trials showed indications of participant 2 and 7 being in a FOR state due to a decrease in 10 km TT performance post-training compared to pre-training with a *super-compensatory* performance increase post-recovery. This was supported by a decrease in the exercise-induced salivary testosterone response and sport stress scores post-training compared to pre-training.

5.3 10 km Time Trial Performance

A hallmark feature of overreaching and the OTS is the decrease in sport-specific performance due to inability to sustain the exercise intensity during increases in training load (Urhausen, Gabriel and Kindermann, 1995; Meeusen *et al.*, 2004). With this being a primary indicator of overreaching, the 12 day intensified training period did have an impact on the participant's performance. As illustrated in Figure 5, the TTC pre-training was 45.2 ± 5.3 min and post-training 46.4 ± 4.7 min. Although the 10 km TT performance did not significantly differ from pre- to post-training, a small ES (0.24) was observed with a performance decrease of 3%. This may only be a small decrease but it is an important finding for trained athletes as this would highlight a meaningful reduction in their performance. Furthermore, it suggests that the 118% increase in training load over a 12 day period induced early stages of overreaching (specifically FOR). Previous studies have also reported a decline in performance of similar magnitude reported in the present study (Jeukendrup *et al.*, 1992; Snyder *et al.*, 1995; Meeusen *et al.*, 2004). Jeukendrup *et al.* (1992) reported a 3-4% decrease in maximal aerobic power during a graded incremental test to exhaustion as well as a 5% decrease in an 8.5 km TT performance on a cycle ergometer following a 2 week period of intensified training. Moreover, Meeusen and colleagues observed decreases in cycling performance (time to voluntary exhaustion) following a 10 day training camp. Specifically, they found a 6% decrease in overreached athletes and an 11% decrease in OTS athletes.

There is further evidence to suggest that the participants in the present study were induced into an overreached state (specifically FOR) as seen in the four participants who completed all three main trials. Although one participant was removed (participant 1) from the data analysis due to not completing the post-training 10 km TT, average performances decreased from pre- to post-training and a *super-compensatory* effect occurred post-recovery. It should be noted that a large *SD* for all trials occurred due to only a small number of participants (n = 3) completing the all three main trials and a large variation between each participants TTC. Although no significant differences were found between all three trials, ES show that a moderate decrease from pre- to post-training (0.76) and a large increase from post-training to post-recovery (0.89) occurred. The calculated ES highlight that the changes that occur in performances following a 12 day intensified training period and 4 day recovery period. Furthermore, TTC results show that participants were able to recover sufficiently following a 4 day recovery period. Previous studies have suggested that well-trained runners need a minimum of 48 h to fully recover from a 10 km race (Gómez *et al.*, 2002).

Further analysis of individual 10 km TT performances have highlighted that participant 2 and 7 both showed similar responses over the duration of the study which indicate signs of being in a FOR state. As a group, overreaching could not be confirmed and therefore participant 2 and 7 were selected for individual analysis due to many of their responses being associated with FOR, for example, a decrease in 10 km TT performance and a blunted hormone (testosterone) response. Participant 2 and 7 10 km TT performances were slower pre- (participant 2: 45.0 min; participant 7: 35.7 min) to post-training (participant 2: 53.2 min; participant 7: 40.0 min) and performances were *super-compensatory* following a 4 day recovery period (participant 2: 41.5 min; participant 7: 34.0 min). This is an indication of both participants being in an FOR state due to post-recovery performance levels increasing beyond pre- and post-training levels. It should be noted that the variation in TTC due to different level of athletes recruited for this study could have an influence on the results. Athletes who were more accustomed to the heavy training load are more likely to cope with feelings of fatigue better compared to recreational athletes. This could lead to larger variations in post-training 10 km TT performances as observed in participant 2 and 7.

5.4 Salivary Testosterone Response

The exercise-induced salivary testosterone response increased significantly by 36% during the pre-training main trial highlighting the capability of the $RPE_{treadmill}$ to induce robust elevations in salivary testosterone when in a normal trained state. This supports the findings of previous work conducted in the University of Bedfordshire's laboratories which was reported that this exercise-induced response is reproducible (Leal, 2017). This finding is also in agreement with Hough *et al.* (2011) and Hough *et al.* (2013) who reported comparable findings in which salivary testosterone were shown to elevate to a protocol of a similar exercise intensity but with a different mode of exercise (a continuous 30 min high-intensity cycling bout referred to as the 55/80) when individuals were in a normal trained state. Furthermore, previous studies have shown that an exercise-induced increase when in a normal trained state is important to highlight any alterations that occur during periods of intensified training (Hough *et al.*, 2011, 2013). Therefore, the importance of the $RPE_{treadmill}$ to induce a significant elevation in salivary testosterone in this study and previous studies is essential to the detection of overreaching. This would help highlight any blunting response which would occur if an athlete was in an overreached state.

The exercise-induced salivary testosterone response did not significantly differ between pre- and post-training, however a significant increase in this variable was found when examined in the pre-training main trial. This could suggest initial stages of a blunted response of salivary testosterone following the 12 day intensified training period. Following the 12 day intensified training period, the exercise-induced salivary testosterone response decreased by 42% when compared to pre-training responses. This is supported by a small ES between pre- and post-training (0.43) which highlights a meaningful reduction between trials. A possible explanation for the initial stages of a blunted salivary testosterone response following an intensified training period could be due to repeated exposure to individual exercise sessions on a daily basis which may have resulted in elevations in cortisol production. It has been suggested that high levels of cortisol may negatively impact upon the testosterone response by disrupting the testicular testosterone biosynthesis pathway (Cumming, Quigley and Yen, 1983). However, blood samples were not collected to confirm whether there was an interaction between these hormones to confirm this theory. This finding is in agreement with previous literature (Hough *et al.*, 2013; Hough, Robertson and Gleeson, 2015). Previous studies have shown the exercise-induced salivary testosterone response to decrease by 44% in a group of elite triathletes following a 10 day intensified training period (Hough, Robertson and Gleeson, 2015) and decrease by 21% in physically active males following a 11 day intensified training period (Hough *et al.*, 2013). The reductions found in these studies are similar to the finding in the present study suggesting that the athletes showing indications of a blunted testosterone response following periods of intensified training could be a potential biological marker for those in the initial stages of overreaching.

It was also hypothesised that the exercise-induced salivary testosterone response would return to, or increase above pre-training levels following a 4 day recovery period. No significant differences were observed between pre-training, post-training and post-recovery. However, ES show a moderate decrease in the exercise-induced salivary testosterone response from pre- to post-training and large increases from post-training to post-recovery and pre-training to post-recovery. These findings support hypothesis four. Further evidence of the participants showing indications of a blunted exercise-induced salivary testosterone response is supported by the individual responses found in participants 2 and 7. Firstly, both participants salivary testosterone response pre-training elevated by 67% (participant 2) and 49% (participant 7) in response to the $RPE_{treadmill}$ bout which is important to highlight any alterations in an overreached state. Following the 12 day intensified training, both participants showed

decreases of 93% and 144% in the exercise-induced salivary testosterone response. This finding supports the view that participant 2 and 7 were in an overreached state. With the primary indicator for overreaching being a decrease in performance which was observed in both participants, the blunted exercise-induced salivary testosterone response may be a biological indicator of overreaching. Furthermore, the post-recovery exercise-induced salivary testosterone responses returned to pre-training levels and therefore it can be specifically viewed as both participants were in an FOR state. This is supported by the post-recovery 10 km TT performance being quicker and sport stress scores decreasing compared to post-training. This supports the view that these two participants are overreached as this highlights a *super-compensatory* response in the exercise performance which is key feature of FOR (Meeusen *et al.*, 2013).

5.5 Salivary Cortisol Response

An unexpected finding from this study was that the exercise-induced salivary cortisol response did not significantly differ from pre- to post-training which is contradictory to hypothesis one. Although no significant differences occurred, a small increase was observed for the exercise-induced salivary cortisol response between pre- to post-training (ES = 0.49) with an increase of 675%. It was also hypothesised that the $RPE_{treadmill}$ would induce an elevation in the salivary cortisol response pre-training when participants were in a normal trained state. Thereafter, a blunted response to the $RPE_{treadmill}$ post-training would occur. However, as illustrated in Figure 9, the $RPE_{treadmill}$ bout failed to induce a significant elevation in cortisol pre-training which is necessary to highlight any alterations which may occur as a result of overreaching (Hough *et al.*, 2011, 2013). Previous studies which have examined the effects of intensified training periods on the exercise-induced hormone response have found cortisol to decrease (Meeusen *et al.*, 2004; Hough *et al.*, 2013). Hough *et al*. (2013) reported a 166% decrease in the exercise-induced salivary cortisol response following an 11 day intensified training period. Moreover, Meeusen *et al.* (2004) found a blunted cortisol response to the second test of a two-bout maximal exercise protocol following a 10 day intensified training period. A possible explanation for this finding found in the present study could be due to unaltered RESTQ-76 Sport questionnaire scores from pre- to post-training which suggests that the participants were able to cope with the increase in training load. This explanation was also suggested by Hough, Robertson and Gleeson

(2015) who also found the exercise-induced salivary cortisol response to be unaltered following a 10 day intensified training camp in elite male triathletes. Another possible explanation could be that the $RPE_{treadmill}$ bout may not have been stressful enough to induce a significant elevation and could suggest that cortisol may not be sensitive enough to indicate an increase in training load.

No significant differences were found across all trials (Figure 10), however small to large ES were observed which suggests that a difference between trials did occur. It was hypothesised a blunted response would occur following a 12 day intensified training period and thereafter salivary cortisol responses would return to, or increase above pre-training levels following a 4 day recovery period. However, contrary to this hypothesis, the ES show that compared to pre-training, cortisol responses increased by small magnitude (0.4) post-training and increased by a large magnitude post-recovery (0.92). The small to large increases found could be due to the large variation in individual responses to the $RPE_{treadmill}$ bout (Figure 21). Analysis of participant 2 and 7 who have been identified as being in a state of FOR also suggests that cortisol may not be most reliable marker of overreaching. The $RPE_{treadmill}$ did not increase the salivary cortisol response in participant 7 when in a normal trained state. In contrast, participant 2 did have exercise-induced increase pre-training and a 100% blunted response post-training. As seen with these two participants, cortisol does seem to produce variable results and it does suggest that salivary testosterone may be the more sensitive marker (compared to cortisol) to an elevation of exercise training stress. This finding is in agreement with Hough, Robertson and Gleeson (2015) findings.

5.6 Salivary Secretory IgA Response

In the present study, SIgA was expressed as absolute concentration and secretion rate. Furthermore, this study examined the resting and exercise-induced responses to the $RPE_{treadmill}$ bout. Expressing SIgA as two different methods produced varying results. Firstly, the resting SIgA absolute concentrations did not significantly differ from pre- to post-training. However, when analysing the four participants which completed the additional post-recovery main trial, a significant decrease from pre- to post-training was observed. This finding is in agreement with previous literature which has reported resting SIgA absolute concentrations to decrease following a period of intensified training (Tharp and Barnes, 1990; Robson *et al.*, 1999; Gleeson *et al.*, 2002; Fahlman and Engels, 2005; Neville, Gleeson and

Folland, 2008). However, this finding may be solely due to the low number of participants which completed all three trials. Direct comparison with some of these studies is difficult due to being conducted over a longer duration (i.e. competitive season) compared to the present study. In comparison to this study, many of these studies have only observed resting SIgA absolute concentrations in response to an athlete's normal training throughout a season and have not intentionally increased the athlete's training load to induce a state of overreaching. However, some studies of a similar nature have shown resting SIgA absolute concentrations to decline following a period of intensified training (Robson *et al.*, 1999; Gleeson *et al.*, 2002). Gleeson *et al.* (2002) reported a reduction in resting SIgA absolute concentrations following a 30 day intensified training period in elite swimmers. Other studies have showed resting SIgA absolute concentrations to decrease in military cadets following a 5 day combat course (Tiollier *et al.*, 2005) and in Australian Air Force pilots following a 19 day survival course (Carins and Booth, 2002). However, other stressors (i.e. psychological stress, hunger and sleep deprivation) were induced within these studies and together with the intensified training would have likely contributed to the reduction in SIgA reported in these studies.

Moreover, the present study investigated the exercise-induced SIgA concentrations and secretion rate to the $RPE_{treadmill}$ bout. It was hypothesised that an exercise-induced SIgA response to the $RPE_{treadmill}$ bout would occur and thereafter, a blunted response would result following a 12 day intensified training period. The findings revealed that SIgA concentrations and secretion rate was unaffected by the exercise and training which is contrary to hypothesis two. Although the $RPE_{treadmill}$ failed to induce a significant elevation, the exercise-induced SIgA secretion rate did decrease by 309% from pre- to post-training. This decrease is supported by the calculated ES which revealed that a small decline (ES = 0.32) occurred for secretion rate. Although this may only represent a small effect, it does highlight that the periods of intensified training have an impact on mucosal immunity. A limited number of studies have investigated the effects of periods of intensified training on the exercise-induced SIgA responses, specifically in response to short-duration, high-intensity exercise. Studies which have, have done so over a 7 month period in response to individual swimming sessions lasting longer than 1.5 h (Gleeson *et al.*, 1995). Studies which have investigated the effect of short-duration, high-intensity exercise on the SIgA responses have shown secretion rates to increase post-exercise (Blannin *et al.*, 1998; Allgrove *et al.*, 2008). Allgrove and colleagues reported SIgA secretion rates to increase following an incremental test to exhaustion and Blannin and colleagues reported SIgA absolute concentrations and secretion rates to increase

following a cycle to exhaustion (~37 min) at 80% $\dot{V}O_{2max}$. These studies highlight that SIgA is elevated to high-intensity exercise which was not found in the present study. A possible explanation for this could be due to the different protocols used in this study. The $RPE_{treadmill}$ consisted of alternating blocks of 1 min at 11 RPE and 4 min at 15 RPE and when calculated was equal to ~67% $\dot{V}O_{2max}$ which may not have been intense enough to induce significant elevations in SIgA. Previous studies have used an exercise protocol where participants sustain a higher exercise intensity (Blannin *et al.*, 1998; Allgrove *et al.*, 2008). For example, Blannin *et al.* (1999) exercise protocol was a cycle to exhaustion at 80% $\dot{V}O_{2max}$(~37 min) and Allgrove *et al.* (2008) was maximal incremental cycle test to exhaustion (~22 min).

It has been suggested that during periods of intensified training, athletes experience illnesses more frequently and therefore SIgA could be used as a marker of overreaching to predict the occurrence of URS (Nieman, 2003). Specifically, the secretion rate of SIgA has been suggested as a unique predictor of URS (Fahlman and Engels, 2005). The present study did not find any significant differences in URS between the pre- and post-training other than headaches were significantly higher from pre- to post-training. However, moderate ES were found between pre- and post-training. This highlights the impact of the 12 day intensified training period on the participants which could suggest that chronic exercise impairs mucosal immunity leading to an increase in illnesses. With this said, the SIgA secretion rate which declined post-training by 309% may have contributed to the increase in URS. However, understanding the causes of the increased occurrence of URS post-training is still unknown as no significant differences were found in the exercise-induced concentrations and secretion rates between pre- and post-training which contradicts the hypothesis previously proposed in this study. Furthermore, the four participants that completed all three main trials did not have any significant differences in their URS scores. As previously mentioned, a small sample size could have attributed to this. In addition, potential explanation for this finding could be due to the number of days it takes for URS to appear following exposure and therefore alterations in SIgA may not have been present in the whole group. It has been suggested that the incubation period of URS is usually 1 to 3 days (Department of Health CDCB, 2005).

As overreaching could not be confirmed as whole group, participant 2 and 7 have been highlighted as being in FOR state. Therefore, it is expected that the a decrease in SIgA and the occurrence of URS is more frequent in overreached athletes (Nieman, 2003). Examining these participants individually could help provide better clarification of the occurrence of

URS in an overreached state. However, the number of URS reported varied between participant 2 and 7. Participant 2 reported no URS pre-training, post-training and post-recovery whereas participant 7 reported four URS post-training (mild to moderate symptoms) and none pre-training and post-recovery. It is possible that the increase in URS reported from participant may have been due to the 114% decrease in exercise-induced SIgA secretion rate. Previous studies have found SIgA responses to be consistently lower by 18-32% in stale athletes and possibly predisposed to OTS over a 6 month competitive season (Mackinnon and Hooper, 1994). However, it cannot be concluded whether the lowering of SIgA secretion rate was responsible for increased URS in participant 7 because SIgA concentrations were increased post-training by 60%. It is clear from the whole group data and the individual data that more research needs to be conducted on the exercise-induced SIgA responses and its response following periods of intensified training.

5.7 Applications of Findings

The present study aimed to establish a useful tool which would highlight any blunted salivary cortisol and testosterone response which would aid in the identification of overreaching (specifically NFOR). The findings of this study suggest that the $RPE_{treadmill}$ bout could be a useful tool in highlighting the initial stages of any alterations in salivary testosterone following a period of intensified training. Therefore, the $RPE_{treadmill}$ bout could be useful for athletes and coaches to help diagnose and prevent the occurrence of overreaching and also monitor training loads to successfully achieve optimal performance. Specifically, the $RPE_{treadmill}$ bout provides as a more practical and alternative test for runners to use compared to the cycling tests used in Hough *et al.* (2013), Hough, Robertson and Gleeson (2015) and Meeusen *et al.* (2004). Furthermore, the advantage of $RPE_{treadmill}$ is its usability in the field (i.e. athletics track) which makes it easy for athletes/coaches to incorporate into their training programmes. The $RPE_{treadmill}$ test is feasible for athletes/coaches to use as it can be performed without the need for any specialist equipment or knowledge. The simplicity of using a Borg scale means exercise intensity of the test can be easily determined as it is down to the individual's own perception of exertion and therefore there is no need for preliminary testing.

Another finding of this study suggests that salivary cortisol may not be a useful marker in the identification of overreaching. In comparison to testosterone, the $RPE_{treadmill}$ bout failed to induce a significant cortisol elevation pre-training which is required to identify any alteration

in this hormone to occur post-training. This could be due to the intensity of the $RPE_{treadmill}$ bout not being high enough and therefore future research should consider increasing the intensity of this bout to induce a significant elevation. This would provide better understanding of salivary cortisol as a marker of overreaching.

Furthermore, the 12 day intensified training period implemented in this study aimed to induce a state of overreaching in these athletes but was not sufficient enough to cause overreaching. As a whole group overreaching could not be confirmed and as highlighted from the individual data, only two participants showed markers which were consistent with overreaching. Although, overreaching could not be confirmed as a whole group, increasing the training load did show to have impact on the individual's performance. This is important for coaches to consider when increasing an athlete's training load.

5.8 Limitations and Future Research

One of the limitations of the present study is the use of the Jackson Score URS questionnaire. Although its use is not a limitation itself as it has been validated against pathogen identification methods and it is regarded as a reliable tool to use in exercise studies (Bermon *et al.*, 2017). But better clarity is needed to understand the impact of an intensified training period on mucosal immune system. Therefore, future research should aim to include regular clinical assessments throughout the study to provide better clarity on URS and other causes of URS (i.e. beyond infection).

The small number of participants recruited for this study resulted in a low power outcome from the post-hoc power analysis which is a limitation of the study. However, due to the nature of the study design, the completion of eight participants requires much work. In addition, the small number of participants that completed the post-recovery main trial was also a limitation. Due to high drop-outs and certain complications throughout the study, some individuals were not able to complete this trial and therefore a true comparison of results between all three main trials was difficult. Future research should aim to increase the number of participants which may provide a better understanding of hormonal and immunological responses following a recovery period after completing a 12 day intensified training period.

Furthermore, another limitation to consider is not measuring for blood contamination of saliva samples in cortisol and testosterone. The detection of blood contamination in SIgA is

not relevant as blood IgA is monomeric. It has been suggested that blood leakage into saliva can occur from accidental injuries, poor oral health or some infectious diseases (Kamodyová *et al.*, 2015; Brescia *et al.*, 2016). Therefore, blood leakage into saliva from the oral mucosa could result in compromised salivary hormone values (Malamud and Tabek, 1993). Future research should aim to reduce the likelihood of this occurring by including a blood contamination measurement of salivary samples into the salivary handling and collection process. One approach would be to systematically inspect each saliva sample at the moment of collection and if any traces of blood are visible to the naked eye should be excluded from the analysis (Brescia *et al.*, 2016).

5.9 Conclusion

Overall, the present study demonstrated that the $RPE_{treadmill}$ significantly induces robust elevations in salivary testosterone when in a normal trained state and therefore this exercise protocol could be used to highlight any alterations when in an overreached state. No significant exercise-induced salivary testosterone response occurred post-training suggesting that the initial stages of a blunted salivary testosterone response occurs following a 12 day intensified training period. Therefore, the $RPE_{treadmill}$ bout could be used as a tool for athletes (specifically runners) and coaches to identify the alterations in salivary testosterone to help diagnose and prevent overreaching in athletes. Moreover, the 10 km TT performance decreased from pre- to post-training which is an important marker to use when diagnosing athletes as overreached. The exercise-induced absolute concentrations and secretion rate of SIgA did not significantly differ following a 12 day intensified training period. However, the small ES for secretion rate does provide some evidence of mucosal immunity to be inhibited. Therefore, it can be concluded that the 12 day intensified training period may have only induced a state of overreaching in some participants and not in all of them. Participant 2 and 7 were individually analysed and were identified as being in an FOR state. A decrease in 10 km TT performance and *super-compensatory* improvement was the primary indicator of this and it was coupled with a blunted salivary testosterone response and sport stress scores. These are all important markers which have been reported in athletes diagnosed as overreached (Meeusen *et al.*, 2013).

References

Allgrove, J. E., Gomes, E., Hough, J. and Gleeson, M. (2008) 'Effects of exercise intensity on salivary antimicrobial proteins and markers of stress in active men', *Journal of Sports Sciences*, 26(6), pp. 653–661.

American College of Sports Medicine (2018) *ACSM's guidelines for exercise testing and prescription, Lippincott Williams & Wilkins*.

Armstrong, L. E. and VanHeest, J. L. (2002) 'The unknown mechanism of the overtraining syndrome: clues from depression and psychoneuroimmunology.', *Sports Medicine*, 32(3), pp. 185–209.

Bamberger, C. M., Schulte, H. M. and Chrousos, G. P. (1996) 'Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids', *Endocrine Reviews*, 17(3), pp. 245–261.

Bermon, S. F., Calder, P. C., Krüger, K. and Senchina, D. (2017) 'Consensus Statement Immunonutrition and Exercise', *Immunonutrition and Exercise*, 23, pp. 8–50.

Birrer, D., Lienhard, D., Williams, C. A., Röthlin, P. and Morgan, G. (2013) 'Prevalence of non-functional overreaching and the overtraining syndrome in Swiss elite athletes', *Schweizerische Zeitschrift fur Sportmedizin und Sporttraumatologie*, 61(4), pp. 23–29.

Bishop, N. C. and Gleeson, M. (2009) 'Acute and chronic effects of exercise on markers of mucosal immunity', *Frontiers in Bioscience*, 14, pp. 4444–4456.

Blannin, A., Robson, P., Walsh, N., Clark, A., Glennon, L. and Gleeson, M. (1998) 'The Effect of Exercising to Exhaustion at Different Intensities on Saliva Immunoglobulin A, Protein and Electrolyte Secretion', *International Journal of Sports Medicine*, 19(8), pp. 547–552.

Borresen, J. and Lambert, M. (2009) 'The quantification of training load, the training response and the effect on performance', *Sports Medicine*, 39(9), pp. 779–795.

Bosch, J. a, Ring, C., de Geus, E. J. C., Veerman, E. C. I. and Amerongen, A. V. N. (2002) 'Stress and secretory immunity', *International Review of Neurobiology*, 52, pp.

213–253.

Brescia, V., Cardinali, R., Zecca, C., Maria, ·, Dell 'abate, T. and Burano, R. (2016) 'Influence of blood contamination on the salivary cortisol level', *Italian Journal of Laboratory Medicine*. Società Italiana di Patologia Clinica e Medicina di Laboratorio, 12(February), pp. 59–61.

Brownlee, K. K., Moore, A. W. and Hackney, A. C. (2005) 'Relationship between circulating cortisol and testosterone: Influence of physical exercise', *Journal of Sports Science and Medicine*, 4(1), pp. 76–83.

Cadore, E., Lhullier, F., Brentano, M., Silva, E., Ambrosini, M., Spinelli, R., Silva, R. and Kruel, L. (2008) 'Correlations between serum and salivary hormonal concentrations in response to resistance exercise', *Journal of Sports Sciences*, 26(10), pp. 1067–1072.

Capen, C. C. (2001) 'Toxic Responses of the Endocrine System', in *Casarett and Doull´s Toxicology, The Basic Science of Poisons*, pp. 711–759.

Carins, J. and Booth, C. (2002) 'Salivary immunoglobulin-A as a marker of stress during strenuous physical training', *Aviation Space and Environmental Medicine*, 73(12), pp. 1203–1207.

CDCB, D. of H. (2005) *You've got what?: prevention and control of notifiable and other infectious diseases in children and adults*. 3rd edn. South Australia: Govt of South Australia.

Chan, S. and Debono, M. (2010) 'Review: Replication of cortisol circadian rhythm: new advances in hydrocortisone replacement therapy', *Therapeutic Advances in Endocrinology and Metabolism*, 1(3), pp. 129–138.

Chidley, C. and Davison, G. (2017) 'The effect of Chlorella pyrenoidosa supplementation on immune responses to 2 days of intensified training', *European Journal of Nutrition*, pp. 1–8.

Chrousos, G. P. (1995) 'The hypothalamic-pituitary-adrenal axis and immune mediated inflammation.', *The New England Journal of Medicine*, 7(332), pp. 1351–62.

Cohen, J. (1988) *Statistical power analysis for the behavioral sciences, Statistical Power*

Analysis for the Behavioral Sciences.

Cohen, J. (1992) 'A power primer', *Psychological Bulletin*, 112(1), pp. 155–159.

Cole, A. E. and Eastoe, J. E. (1988) *Biochemistry and Oral Biology*. 2nd edn. Butterworth-Heinemann.

Collomp, K., Arlettaz, A., Portier, H., Lecoq, A. M., Le Panse, B., Rieth, N. and De Ceaurriz, J. (2008) 'Short-term glucocorticoid intake combined with intense training on performance and hormonal responses', *British Journal of Sports Medicine*, 42(12), pp. 983–988.

Constantini, N. and Hackney, A. C. (2013) *Endocrinology of Physical Activity and Sport*. 2nd edn. New York: Humana Press.

Cox, A. J., Gleeson, M., Pyne, D. B., Callister, R., Hopkins, W. G. and Fricker, P. A. (2008) 'Clinical and laboratory evaluation of upper respiratory symptoms in elite athletes', *Clinical Journal of Sport Medicine*, 18(5), pp. 438–445.

Crewther, B. T., Lowe, T. E., Ingram, J. and Weatherby, R. P. (2010) 'Validating the salivary testosterone and Cortisol concentration measures in response to short high-intensity exercise', *Journal of Sports Medicine and Physical Fitness*, 50(1), pp. 85–92.

Cumming, D. C., Quigley, M. E. and Yen, S. S. C. (1983) 'Acute suppression of circulating testosterone levels by cortisol in men', *Journal of Clinical Endocrinology and Metabolism*, 57(3), pp. 671–673.

Cumming, D. C. and Wali, S. R. (1985) 'Non-sex hormone-binding globulin-bound testosterone as a marker for hyperandrogenism', *Journal of Clinical Endocrinology and Metabolism*, 61(5), pp. 873–876.

Czeisler, C. A., Johnson, M. P., Duffy, J. F., Brown, E. N., Ronda, J. M. and Kronauer, R. E. (1990) 'Exposure to Bright Light and Darkness to Treat Physiologic Maladaptation to Night Work', *New England Journal of Medicine*, 322(18), pp. 1253–1259.

Davies, C. T. M. and Few, J. D. (1973) 'Effects of exercise on adrenocortical function', *Journal of Applied Physiology*, 35(6), pp. 887–891.

Debono, M., Ghobadi, C., Rostami-Hodjegan, A., Huatan, H., Campbell, M. J., Newell-Price,

J., Darzy, K., Merke, D. P., Arlt, W. and Ross, R. J. (2009) 'Modified-release hydrocortisone to provide circadian cortisol profiles', *Journal of Clinical Endocrinology and Metabolism*, 94(5), pp. 1548–1554.

Department of Health Physical Activity Health Improvement and Protection (2011) *Start Active, Stay Active: A report on physical activity from the four home countries' Chief Medical Officers, Report.*

Dhabhar, F. S. (2009) 'Enhancing versus suppressive effects of stress on immune function: Implications for immunoprotection and immunopathology', *NeuroImmunoModulation*, 16(5), pp. 300–317.

Dimitriou, L., Sharp, N. C. C. and Doherty, M. (2002) 'Circadian effects on the acute responses of salivary cortisol and IgA in well trained swimmers.', *British Journal of Sports Medicine*, 36(4), pp. 260–264.

Dorrington, M., Gleeson, M. and Callister, R. (2003) 'Effect of exercise intensity on salivary IgA in Children', *Journal of Science and Medicine in Sport*, 6(4), p. 46.

Duclos, M. (2008) 'A critical assessment of hormonal methods used in monitoring training status in athletes', *International SportMed Journal Official Journal of FIMS (International Federation of Sports Medicine) International SportMed Journal*, 99(22), pp. 56–6656.

Duclos, M., Corcuff, J. B., Rashedi, M., Foug??re, V. and Manier, G. (1997) 'Trained versus untrained men: Different immediate post-exercise responses of pituitary adrenal axis. A preliminary study', *European Journal of Applied Physiology and Occupational Physiology*, 75(4), pp. 343–350.

Duclos, M., Gouarne, C. and Bonnemaison, D. (2003) 'Acute and chronic effects of exercise on tissue sensitivity to glucocorticoids.', *Journal of Applied Physiology*, 94(3), pp. 869–875.

Duplessis, C., Rascona, D., Cullum, M. and Yeung, E. (2010) 'Salivary and free serum cortisol evaluation.', *Military Medicine*, 175(5), pp. 340–6.

Engler, D., Pham, T., Fullerton, M. J., Ooi, G., Funder, J. W. and Clarke, I. J. (1989) 'Studies of the secretion of corticotropin-releasing factor and arginine vasopressin into the

hypophysial-portal circulation of the conscious sheep: I. effect of an audiovisual stimulus and insulin-induced hypoglycemia', *Neuroendocrinology*, 49(4), pp. 367–381.

Fahlman, M. M. and Engels, H.-J. J. (2005) 'Mucosal IgA and URTI in American college football players: a year longitudinal study.', *Medicine and Science in Sports and Exercise*, 37(3), pp. 374–80.

Filaire, E., Legrand, B., Lac, G. and Pequignot, J.-M. (2004) 'Training of elite cyclists: effects on mood state and selected hormonal responses.', *Journal of Sports Sciences*, 22(11–12), pp. 1025–1033.

Flynn, M. G., Pizza, F. X., Boone, J. B., Andres, F. F., Michaud, T. A. and Rodriguez-Zayas, J. R. (1994) 'Indices of training stress during competitive running and swimming seasons', *International Journal of Sports Medicine*, 15(1), pp. 21–26.

Fries, E., Dettenborn, L. and Kirschbaum, C. (2009) 'The cortisol awakening response (CAR): Facts and future directions', *International Journal of Psychophysiology*, 72(1), pp. 67–73.

Fry, R. W., Grove, J. R., Morton, A. R., Zeroni, P. M., Gaudieri, S. and Keast, D. (1994) 'Psychological and immunological correlates of acute overtraining', *British Journal of Sports Medicine*, 28(4), pp. 241–246.

Gleeson, M. (2000) 'Mucosal immune responses and risk of respiratory illness in elite athletes.', *Exercise Immunology Review*, 6(6), pp. 5–42.

Gleeson, M. and Bishop, N. C. (2013) 'URI in Athletes : Are Mucosal Immunity and Cytokine Responses Key Risk Factors?', *Exercise and Sport Sciences Reviews*, 41(10), pp. 2–7.

Gleeson, M., Bishop, N. C. and Walsh, N. P. (2013) *Exercise Immunology*. 1st edn. Routledge.

Gleeson, M., Bishop, N., Oliveira, M., McCauley, T., Tauler, P. and Muhamad, A. S. (2012) 'Respiratory infection risk in athletes: Association with antigen-stimulated IL-10 production and salivary IgA secretion', *Scandinavian Journal of Medicine and Science in Sports*, 22(3), pp. 410–417.

Gleeson, M., Ginn, E. and Francis, J. L. (2000) 'Salivary immunoglobulin monitoring in an elite kayaker', *Clinical Journal of Sport Medicine*, 10(3), pp. 206–208.

Gleeson, M., McDonald, W. a, Cripps, a W., Pyne, D. B., Clancy, R. L. and Fricker, P. a (1995) 'The effect on immunity of long-term intensive training in elite swimmers.', *Clinical and Experimental Immunology*, 102(1), pp. 210–216.

Gleeson, M. and Pyne, D. B. (2000) 'Exercise effects on mucosal immunity', *Immunology and Cell Biology*, 78(5), pp. 536–544.

Gleeson, M. and Pyne, D. B. (2000) 'Special feature for the Olympics: effects of exercise on the immune system: exercise effects on mucosal immunity', *Immunology and Cell Biology*, 78(5), pp. 536–544.

Gleeson, M., Pyne, D. B., Austin, J. P., Lynn Francis, J., Clancy, R. L., McDonald, W. a and Fricker, P. a (2002) 'Epstein-Barr virus reactivation and upper-respiratory illness in elite swimmers.', *Medicine and Science in Sports and Exercise*, 34(3), pp. 411–417.

Gomez-Merino, D., Drogou, C., Chennaoui, M., Tiollier, E., Mathieu, J. and Guezennec, C. Y. (2005) 'Effects of combined stress during intense training on cellular immunity, hormones and respiratory infections', *NeuroImmunoModulation*, 12(3), pp. 164–172.

Gómez, A. L., Radzwich, R. J., Denegar, C. R., Volek, J. S., Rubin, M. R., Bush, J. A., Doan, B. K., Wickham, R. B., Mazzetti, S. A., Newton, R. U., French, D. N., Häkkinen, K., Ratamess, N. A. and Kraemer, W. J. (2002) 'The effects of a 10-kilometer run on muscle strength and power', *Journal of Strength and Conditioning Research*, 16(2), pp. 184–191.

Halson, S. L. (2014) 'Monitoring Training Load to Understand Fatigue in Athletes', *Sports Medicine*, 44(2), pp. 139–147.

Halson, S. L., Bridge, M. W., Meeusen, R., Busschaert, B., Gleeson, M., Jones, D. A. and Jeukendrup, A. E. (2002) 'Time course of performance changes and fatigue markers during intensified training in trained cyclists.', *Journal of Applied Physiology*, 93(3), pp. 947–956.

Halson, S. L. and Jeukendrup, A. E. (2004) ‘Does overtraining exist? An analysis of overreaching and overtraining research’, *Sports Medicine*, 34(14), pp. 967–981.

Halson, S. L., Lancaster, G. I., Jeukendrup, A. E. and Gleeson, M. (2003) ‘Immunological responses to overreaching in cyclists’, *Medicine and Science in Sports and Exercise*, 35(5), pp. 854–861.

Hanstock, H. G., Walsh, N. P., Edwards, J. P., Fortes, M. B., Cosby, S. L., Nugent, A., Curran, T., Coyle, P. V., Ward, M. D. and Yong, X. H. A. (2016) ‘Tear Fluid SIgA as a Noninvasive Biomarker of Mucosal Immunity and Common Cold Risk’, *Medicine and Science in Sports and Exercise*, 48(3), pp. 569–577.

Hastings, M., O’Neill, J. S. and Maywood, E. S. (2007) ‘Circadian clocks: Regulators of endocrine and metabolic rhythms’, *Journal of Endocrinology*, 195(2), pp. 187–198.

Hayes, L. D., Bickerstaff, G. F. and Baker, J. S. (2010) ‘Interactions of Cortisol, Testosterone, and Resistance Training: Influence of Circadian Rhythms’, *Chronobiology International*, 27(4), pp. 675–705.

Helenius, I., Lumme, A. and Haahtela, T. (2005) ‘Asthma, airway inflammation and treatment in elite athletes’, *Sports Medicine*, 35(7), pp. 565–574.

Hill, E. E., Zacki, E., Battaglini, C., Viru, M., Viru, A. and Hackney, A. C. (2008) ‘Exercise and circulating cortisol levels: The intensity threshold effect’, *Journal of Endocrinological Investigation*, 31(7), pp. 587–591.

Hinson, J., Raven, P. and Chew, S. (2010) *The Endocrine System*. Second Edi. Churchill Livingstone Elsevier.

Hooper, S. L., Mackinnon, L. T. and Hanrahan, S. (1997) ‘Mood States as an indication of staleness and recovery’, *International Journal of Sport Psychology*, 28(1), pp. 1–12.

Hooper, S. L., Mackinnon, L. T., Howard, A., Gordon, R. D. and Bachmann, A. W. (1995) ‘Markers for monitoring overtraining and recovery.’, *Medicine and Science in Sports and Exercise*, 27(1), pp. 106–12.

Hough, J., Corney, R., Kouris, A. and Gleeson, M. (2013) ‘Salivary cortisol and testosterone responses to high-intensity cycling before and after an 11-day intensified training

period.', *Journal of Sports Sciences*, 31(14), pp. 1614–23.

Hough, J. P., Papacosta, E., Wraith, E. and Gleeson, M. (2011) 'Plasma and salivary steroid hormone responses of men to high-intensity cycling and resistance exercise.', *Journal of Strength and Conditioning Research / National Strength & Conditioning Association*, 25(1), pp. 23–31.

Hough, J., Robertson, C. and Gleeson, M. (2015) 'Blunting of Exercise-Induced Salivary Testosterone in Elite-Level Triathletes with a 10-Day Training Camp', *International Journal of Sports Physiology and Performance*, 10(7), pp. 935–938.

Ishigaki, T., Koyama, K., Tsujita, J., Tanaka, N., Hori, S. and Oku, Y. (2005) 'Plasma leptin levels of elite endurance runners after heavy endurance training.', *Journal of Physiological Anthropology and Applied Human Science*, 24(6), pp. 573–578.

Jackson, G. G., Dowling, H. F., Spiesman, I. G. and Boand, A. V. (1958) 'Transmission of the Common Cold to Volunteers Under Controlled Conditions: I. The Common Cold as a Clinical Entity', *A.M.A Archives of Internal Medicine*, 101(2), pp. 267–278.

Jafarzadeh, A., Sadeghi, M., Karam, G. A. and Vazirinejad, R. (2010) 'Salivary IgA and IgE levels in healthy subjects: relation to age and gender', *Brazilian Oral Research*, 24(1), pp. 21–27.

Jeukendrup, A. E., Hesselink, M. K., Snyder, A. C., Kuipers, H. and Keizer, H. A. (1992) 'Physiological changes in male competitive cyclists after two weeks of intensified training', *International Journal of Sports Medicine*, 13(7), pp. 534–541.

Johansen, F. E., Braathen, R. and Brandtzaeg, P. (2001) 'The J chain is essential for polymeric Ig receptor-mediated epithelial transport of IgA.', *Journal of Immunology*, 167(9), pp. 5185–5192.

Jones, A. M. and Doust, J. H. (1996) 'A 1% treadmill grade most accurately reflects the energetic cost of outdoor running.', *Journal of Sports Sciences*, 14(4), pp. 321–327.

Jones, A. W., Thatcher, R., March, D. S. and Davison, G. (2015) 'Influence of 4 weeks of bovine colostrum supplementation on neutrophil and mucosal immune responses to prolonged cycling', *Scandinavian Journal of Medicine and Science in Sports*,

25(6), pp. 788–796.

Kamodyová, N., Baňasová, L., Janšáková, K., Koborová, I., Tóthová, ubomíra, Stanko, P. and Celec, P. (2015) 'Blood Contamination in Saliva: Impact on the Measurement of Salivary Oxidative Stress Markers', *Disease Markers*, 2015.

Kellmann, M. and Günther, K. D. (2000) 'Changes in stress and recovery in elite rowers during preparation for the Olympic Games', *Medicine and Science in Sports and Exercise*, 32(3), pp. 676–683.

Kim, H. H. (2007) 'Regulation of gonadotropin-releasing hormone gene expression', *Seminars in Reproductive Medicine*, 25(5), pp. 313–325.

Koch, A. J. (2010) 'Immune Response to Resistance Exercise', *American Journal of Lifestyle Medicine*, 4(3), pp. 244–252.

Kraemer, W. J., Häkkinen, K., Newton, R. U., McCormick, M., Nindl, B. C., Volek, J. S., Gotshalk, L. A., Fleck, S. J., Campbell, W. W., Gordon, S. E., Farrell, P. A. and Evans, W. J. (1998) 'Acute hormonal responses to heavy resistance exercise in younger and older men', *European Journal of Applied Physiology and Occupational Physiology*, 77(3), pp. 206–211.

Kraemer, W. J. and Ratamess, N. A. (2005) 'Hormonal responses and adaptations to resistance exercise and training', *Sports Medicine*, 35(4), pp. 339–361.

Kreher, J. B. (2016) 'Diagnosis and prevention of overtraining syndrome : an opinion on education strategies', *Journal of Sports Medicine*, 6(7), pp. 115–122.

Krieger, D. T., Allen, W., Rizzo, F. and Krieger, H. P. (1971) 'Characterization of the normal temporal pattern of plasma corticosteroid levels', *Journal of Clinical Endocrinology and Metabolism*, 32(2), pp. 266–284.

Kudielka, B. M. and Kirschbaum, C. (2005) 'Sex differences in HPA axis responses to stress: A review', *Biological Psychology*, 69(1), pp. 113–132.

Kvorning, T., Andersen, M., Brixen, K., Schjerling, P., Suetta, C. and Madsen, K. (2007) 'Suppression of testosterone does not blunt mRNA expression of myoD, myogenin, IGF, myostatin or androgen receptor post strength training in humans', *The Journal of Physiology*, 578(Pt 2), pp. 579–593.

Lamm, M. E. (1998) ‘Current concepts in mucosal immunity. IV. How epithelial transport of IgA antibodies relates to host defense.’, *The American Journal of Physiology*, 274(4 Pt 1), pp. G614-7.

Laudat, M. H., Cerdas, S., Fournier, C., Guiban, D., Guilhaume, B. and Luton, J. P. (1988) ‘Salivary cortisol measurement: A practical approach to assess pituitary-adrenal function’, *Journal of Clinical Endocrinology and Metabolism*.

Leal, D. (2017) *The use of acute responses of endocrine and immune biomarkers to highlight overreaching*. University of Bedfordshire.

Leicht, C. A., Bishop, N. C. and Goosey-Tolfrey, V. L. (2011) ‘Mucosal immune responses to treadmill exercise in elite wheelchair athletes’, *Medicine and Science in Sports and Exercise*, 43(8), pp. 1414–1421.

Libicz, S., Mercier, B., Bigou, N., Le Gallais, O. and Castex, F. (2006) ‘Salivary IgA response of triathletes participating in the French Iron Tour’, *International Journal of Sports Medicine*, 27(5), pp. 389–394.

Lippi, G., De Vita, F., Salvagno, G. L., Gelati, M., Montagnana, M. and Guidi, G. C. (2009) ‘Measurement of morning saliva cortisol in athletes’, *Clinical Biochemistry*, 42(9), pp. 904–906.

Luger, A., Deuster, P. A., Kyle, S. B., Gallucci, W. T., Montgomery, L. C., Gold, P. W., Loriaux, D. L. and Chrousos, G. P. (1987) ‘Acute hypothalamic-pituitary-adrenal responses to the stress of treadmill exercise. Physiologic adaptations to physical training.’, *The New England Journal of Medicine*, 316(21), pp. 1309–15.

Mackinnon, L. T. (1996) ‘Immunoglobulin, antibody, and exercise’, *Exercise Immunology Review*, 2, pp. 1–35.

Mackinnon, L. T. (1999) *Advances in Exercise Immunolgy*. Human Kinetics.

MacKinnon, L. T. (2000) ‘Overtraining effects on immunity and performance in athletes’, *Immunology and Cell Biology*, 78(5), pp. 502–509.

Mackinnon, L. T. and Hooper, S. (1994) ‘Mucosal (secretory) immune system responses to exercise of varying intensity and during overtraining.’, *International Journal of Sports Medicine*, 15(3), pp. S179-83.

MacKinnon, L. T. and Jenkins, D. G. (1993) 'Decreased salivary immunoglobulins after intense interval exercise before and after training', *Medicine and science in sports and exercise*, 25(6), pp. 678–683.

McCaulley, G. O., McBride, J. M., Cormie, P., Hudson, M. B., Nuzzo, J. L., Quindry, J. C. and Travis Triplett, N. (2009) 'Acute hormonal and neuromuscular responses to hypertrophy, strength and power type resistane exercise', *European Journal of Applied Physiology*, 105(5), pp. 695–704.

McKeever, K. H. (2002) 'The endocrine system and the challenge of exercise', *Veterinary Clinics of North America - Equine Practice*, 18(2), pp. 321–353.

Meeusen, R., Duclos, M., Foster, C., Fry, A., Gleeson, M., Nieman, D., Raglin, J., Rietjens, G., Steinacker, J. and Urhausen, A. (2013) 'Prevention, diagnosis, and treatment of the overtraining syndrome: Joint consensus statement of the european college of sport science and the American College of Sports Medicine', *Medicine and Science in Sports and Exercise*, 45(1), pp. 186–205.

Meeusen, R., Duclos, M., Gleeson, M., Rietjens, G., Steinacker, J. and Urhausen, A. (2006) 'Prevention, diagnosis and treatment of the Overtraining Syndrome : ECSS Position Statement "Task Force"', *European Journal of Sport Science*, 6(1), pp. 1–14

Meeusen, R., Nederhof, E., Buyse, L., Roelands, B., de Schutter, G. and Piacentini, M. F. (2010) 'Diagnosing overtraining in athletes using the two-bout exercise protocol.', *British Journal of Sports Medicine*, 22(4), pp. 99–105.

Meeusen, R., Piacentini, M. F., Busschaert, B., Buyse, L., De Schutter, G. and Stray-Gundersen, J. (2004) 'Hormonal responses in athletes: The use of a two bout exercise protocol to detect subtle differences in (over)training status', *European Journal of Applied Physiology*, 91(2–3), pp. 140–146.

Miller, W. L. (1988) 'Molecular biology of steroid hormone synthesis.', *Endocrine Reviews*, 9(3), pp. 295–318.

Minetto, M. A., Lanfranco, F., Baldi, M., Termine, A., Kuipers, H., Ghigo, E. and Rainoldi, A. (2007) 'Corticotroph axis sensitivity after exercise: Comparison between elite athletes and sedentary subjects', *Journal of Endocrinological Investigation*, 30(3),

pp. 215–223.

Mrosovsky, N., Reebs, S. G., Honrado, G. I. and Salmon, P. A. (1989) ‘Behavioural entrainment of circadian rhythms’, *Experientia*.

Mujika, I. (2010) ‘Intense training: The key to optimal performance before and during the taper’, *Scandinavian Journal of Medicine and Science in Sports*, 20(SUPPL. 2), pp. 24–31.

Nederhof, E., Zwerver, J., Brink, M., Meeusen, R. and Lemmink, K. (2008) ‘Different diagnostic tools in nonfunctional overreaching’, *International Journal of Sports Medicine*, 29(7), pp. 590–597.

Neville, V., Gleeson, M. and Folland, J. P. (2008) ‘Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes’, *Medicine and Science in Sports and Exercise*, 40(7), pp. 1228–1236.

Nicolaides, N. C., Charmandari, E., Chrousos, G. P. and Kino, T. (2014) ‘Circadian endocrine rhythms: The hypothalamic-pituitary-adrenal axis and its actions’, *Annals of the New York Academy of Sciences*, 1318(1), pp. 71–80.

Nieman, D. C. (1994a) ‘Exercise, infection, and immunity.’, *International Journal of Sports Medicine*, 15 Suppl 3(5), pp. S131–S141.

Nieman, D. C. (1994b) ‘Exercise, upper respiratory tract infection, and the immune system’, *Medicine & Science in Sports & Exercise*, 26(2), pp. 128–139.

Nieman, D. C. (2003) ‘Current perspective on exercise immunology.’, *Current Sports Medicine Reports*, 2(5), pp. 239–242.

Nieman, D. C., Henson, D. A., Fagoaga, O. R., Utter, A. C., Vinci, D. M., Davis, J. M. and Nehlsen-Cannarella, S. L. (2002) ‘Change in salivary IgA following a competitive marathon race’, *International Journal of Sports Medicine*, 23(1), pp. 69–75.

Niu, S. F., Chung, M. H., Chu, H., Tsai, J. C., Lin, C. C., Liao, Y. M., Ou, K. L., O’Brien, A. P. and Chou, K. R. (2015) ‘Differences in cortisol profiles and circadian adjustment time between nurses working night shifts and regular day shifts: A prospective longitudinal study’, *International Journal of Nursing Studies*.

O'Connor, P. J. and Corrigan, D. L. (1987) 'Influence of short-term cycling on salivary cortisol levels.', *Medicine & Science in Sports & Exercise*, 19(3), pp. 224–228.

Oliver, S. J., Laing, S. J., Wilson, S., Bilzon, J. L. J., Walters, R. and Walsh, N. P. (2007) 'Salivary immunoglobulin A response at rest and after exercise following a 48 h period of fluid and/or energy restriction.', *The British Journal of Nutrition*, 97(6), pp. 1109–16.

Owen, A. L., Wong, D. P., Dunlop, G., Groussard, C., Kebsi, W., Dellal, A., Morgans, R. and Zouhal, H. (2016) 'High-Intensity Training and Salivary Immunoglobulin A Responses in Professional Top-Level Soccer Players: Effect of Training Intensity.', *Journal of Strength and Conditioning Research / National Strength & Conditioning Association*, 30(9), pp. 2460–9.

Paccotti, P., Minetto, M., Terzolo, M., Ventura, M., Ganzit, G. P., Borrione, P., Termine, A. and Angeli, A. (2005) 'Effects of high-intensity isokinetic exercise on salivary cortisol in athletes with different training schedules: Relationships to serum cortisol and lactate', *International Journal of Sports Medicine*, 26(9), pp. 747–755.

Palmer, F. M., Nieman, D. C., Henson, D. a, McAnulty, S. R., McAnulty, L., Swick, N. S., Utter, A. C., Vinci, D. M. and Morrow, J. D. (2003) 'Influence of vitamin C supplementation on oxidative and salivary IgA changes following an ultramarathon.', *European Journal of Applied Physiology*, 89(1), pp. 100–107.

Pardridge, W. M. (1986) 'Serum bioavailability of sex steroid hormones.', *Clinics in Endocrinology and Metabolism*, 15(2), pp. 259–278.

Pedersen, B. K. and Ullum, H. (1994) 'NK cell response to physical activity: possible mechanisms of action.', *Medicine & Science in Sports & Exercise*, 26(2), pp. 140–146.

Pittendrigh, C. S. (1993) 'Temporal Organization: Reflections of a Darwinian Clock-Watcher', *Annual Review of Physiology*.

Plisk, S. and Stone, M. (2003) 'Periodization Strategies.', *Strength & Conditioning Journal*, 25(6), pp. 19–37.

Port, K. (1991) 'Serum and saliva cortisol responses and blood lactate accumulation during

incremental exercise testing.', *International Journal of Sports Medicine*, 12(5), pp. 490–4.

Pyne, D. B., McDonald, W. A., Gleeson, M., Flanagan, A., Clancy, R. L. and Fricker, P. A. (2001) 'Mucosal immunity, respiratory illness, and competitive performance in elite swimmers', *Medicine & Science in Sports & Exercise*, 33(3), pp. 348–353.

Reid, V. L., Gleeson, M., Williams, N. and Clancy, R. L. (2004) 'Clinical investigation of athletes with persistent fatigue and/or recurrent infections', *British Journal of Sports Medicine*, 38(1), pp. 42–45.

Reilly, T. and Waterhouse, J. (2009) 'Sports performance: Is there evidence that the body clock plays a role?', *European Journal of Applied Physiology*, 106(3), pp. 321–332.

Rilling, J. K., Worthman, C. M., Campbell, B. C., Stallings, J. F. and Mbizva, M. (1996) 'Ratios of plasma and salivary testosterone throughout puberty: Production versus bioavailability', *Steroids*, 61(6), pp. 374–378.

Robson, P. J., Blannin, A. K., Walsh, N. P., Castell, L. M. and Gleeson, M. (1999) 'The effect of an acute period of intense interval training on human neutrophil function and plasma glutamine in endurance-trained male runners', *Journal of Physiology*, 515P, pp. 84–85.

Sannikka, E., Terho, P., Suominen, J. and Santti, R. (1983) 'Testosterone concentrations in human seminal plasma and saliva and its correlation with non-protein-bound and total testosterone levels in serum', *International Journal of Andrology*, 6(4), pp. 319–330.

Sari-Sarraf, V., Reilly, T., Doran, D. A. and Atkinson, G. (2007) 'The effects of single and repeated bouts of soccer-specific exercise on salivary IgA', *Archives of Oral Biology*, 52(6), pp. 526–532.

Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J. and Stachenfeld, N. S. (2007) 'Exercise and fluid replacement', *Medicine and Science in Sports and Exercise*, 39(2), pp. 377–390.

Saxon, A., Stevens, R. H., Ramer, S. J., Clements, P. J. and Yu, D. T. (1978)

‘Glucocorticoids administered in vivo inhibit human suppressor T lymphocyte function and diminish B lymphocyte responsiveness in in vitro immunoglobulin synthesis’, *Journal of Clinical Investigation*, 61(4), pp. 922–930.

Sayers, G. (1950) ‘The Adrenal Cortex and Homeostasis’, *Physiological Reviews*, 30(3), p. 241 LP-320.

Shephard, R. J. (2010) ‘Development of the discipline of exercise immunology’, *Exercise Immunology Review*, 16, pp. 194–222.

Shirakawa, T., Mitome, M. and Oguchi, H. (2004) ‘Circadian rhythms of S-IgA and cortisol in whole saliva —Compensatory mechanism of oral immune system for nocturnal fall of saliva secretion—’, *Pediatric Dental Journal*, 14(1), pp. 115–120.

Shirtcliff, E. A., Granger, D. A. and Likos, A. (2002) ‘Gender Differences in the Validity of Testosterone Measured in Saliva by Immunoassay’, *Hormones and Behavior*, 42(1), pp. 62–69.

Slivka, D. R., Hailes, W. S., Cuddy, J. S. and Ruby, B. C. (2010) ‘Effects of 21 days of intensified training on markers of overtraining’, *Journal of Strength and Conditioning Research*, 24(10), pp. 2604–2612.

Smith, L. L. (2003) ‘Overtraining, excessive exercise, and altered immunity: Is this a T helper-1 versus T helper-2 lymphocyte response?’, *Sports Medicine*, 33(5), pp. 347–364.

Smith, S. M. and Vale, W. W. (2006) ‘The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress’, *Dialogues in Clinical Neuroscience*, 8(4), pp. 383–395.

Snyder, a C., Kuipers, H., Cheng, B., Servais, R. and Fransen, E. (1995) ‘Overtraining following intensified training with normal muscle glycogen.’, *Medicine and Science in Sports and exercise*, 27(7), pp. 1063–70.

Tharp, G. D. and Barnes, M. W. (1990) ‘Reduction of saliva immunoglobulin levels by swim training.’, *European Journal of Applied Physiology and Occupational Physiology*, 60(1), pp. 61–64.

Tiollier, E., Gomez-Merino, D., Burnat, P., Jouanin, J. C., Bourrilhon, C., Filaire, E.,

Guezennec, C. Y. and Chennaoui, M. (2005) ‘Intense training: Mucosal immunity and incidence of respiratory infections’, *European Journal of Applied Physiology*, 93(4), pp. 421–428.

Tomasi, T. B., Trudeau, F. B., Czerwinski, D. and Erredge, S. (1982) ‘Immune parameters in athletes before and after strenuous exercise’, *Journal of Clinical Immunology*, 2(3), pp. 173–178.

Torpy, D. J. and Ho, J. T. (2007) ‘Value of free cortisol measurement in systemic infection’, *Hormone and Metabolic Research*, 39(6), pp. 439–444.

Tsigos, C. and Chrousos, G. P. (2002) ‘Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress’, *Journal of Psychosomatic Research*, 53(4), pp. 865–871.

Tsujita, S. and Morimoto, K. (1999) ‘Secretory IgA in saliva can be a useful stress marker.’, *Environmental Health and Preventive Medicine*, 4(1), pp. 1–8.

Turnball, A. V. and Rivier, C. L. (1999) ‘Regulation of the Hypothalamic-Pituitary-Adrenal Axis by Cytokines: Actions and Mechanisms of Action’, *Physiological Reviews*, 79(1), pp. 1–71.

Urhausen, a, Gabriel, H. H. and Kindermann, W. (1998) ‘Impaired pituitary hormonal response to exhaustive exercise in overtrained endurance athletes.’, *Medicine and Science in Sports and Exercise*, 30(3), pp. 407–414.

Urhausen, A., Gabriel, H. and Kindermann, W. (1995) ‘Blood Hormones as Markers of Training Stress and Overtraining’, *Sports Medicine*, 20(4), pp. 251–276.

Vingren, J. L., Kraemer, D. W. J., Ratamess, N. A., Anderson, J. M., Volek, J. S. and Maresh, C. M. (2012) ‘Testosterone Physiology in Resistance Exercise and Training’, *Sports Medicine*, 40(12), pp. 1037–1053.

Vining, R. F., McGinley, R. A., Maksvytis, J. J. and Ho, K. Y. (1983) ‘Salivary Cortisol: A Better Measure of Adrenal Cortical Function than Serum Cortisol’, *Annals of Clinical Biochemistry: An International Journal of Biochemistry and Laboratory Medicine*, 20(6), pp. 329–335.

Vittek, J., L’Hommedieu, D. G., Gordon, G. G., Rappaport, S. C. and Southren, A. L. (1985) ‘Direct radioimmunoassay (RIA) of salivary testosterone, correlation with free and

total serum testosterone', *Life Sciences*, 37(8), pp. 711–716.

Walsh, N. P., Bishop, N. C., Blackwell, J., Wierzbicki, S. G. and Montague, J. C. (2002) 'Salivary IgA response to prolonged exercise in a cold environment in trained cyclists', *Medicine & Science in Sports & Exercise*, 34(10), pp. 1632–1637.

Walsh, N. P., Gleeson, M., Shephard, R. J., Gleeson, M., Woods, J. A., Bishop, N. C., Fleshner, M., Green, C., Pedersen, B. K., Hoffman-Goetz, L., Rogers, C. J., Northoff, H., Abbasi, A. and Simon, P. (2011) 'Position statement part one: Immune function and exercise', *Exercise Immunology Review*, 17, pp. 6–63.

Walsh, N. P. and Oliver, S. J. (2016) 'Exercise, immune function and respiratory infection: An update on the influence of training and environmental stress', *Immunology and Cell Biology*, 94(2), pp. 132–139.

Wang, C., Plymate, S., Nieschlag, E. and Alvin Paulsen, C. (1981) 'Salivary testosterone in men: Further evidence of a direct correlation with free serum testosterone', *Journal of Clinical Endocrinology and Metabolism*, 53(5), pp. 1021–1024.

Wilkerson, J. E., Horvath, S. M. and Gutin, B. (1980) 'Plasma testosterone during treadmill exercise.', *Journal of Applied Physiology*, 49(2), pp. 249–253.

Wira, C. R., Sandoe, C. P. and Steele, M. G. (1990) 'Glucocorticoid regulation of the humoral immune system. I. In vivo effects of dexamethasone on IgA and IgG in serum and at mucosal surfaces', *The Journal of Immunology*, 144(1), pp. 142–146.

Woof, J. M. and Ken, M. A. (2006) 'The function of immunoglobulin A in immunity', *Journal of Pathology*, 208(2), pp. 270–282.

Appendices

Appendix A

University of Bedfordshire

Participant Information Sheet

Study Title:

Exercise-induced salivary hormones and immunological responses before and after a 12-day period of intensified-training: preventing the incidence of non-functional overreaching / the overtraining syndrome

Dear participant,

Thank you for showing an interest in taking part in this research study. Please, read this information sheet carefully before deciding whether to participate. If you freely decide to volunteer you are more than welcome and we thank you for your participation. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request. Nevertheless, if you wish at any stage to withdraw from the study there will be no reprisal. Every precaution will be taken to protect the privacy of all the participants and data confidentiality and anonymity will be preserved.

What is the aim of the project?

The purpose of the study is to analyse whether a 12-day period of intensified training may induce a state of overreaching, and to also examine the exercise-induced stress hormone and immune responses to a short-duration, self-paced running bout before and after this 12-day intensified-training period. The use of this exercise tool may be a good method to predict and/or prevent the incidence of overreaching or the overtraining syndrome (OTS), and may be used in any sports-related setting. Additionally, it is intended to examine whether a 4-day recovery period would be enough to normalize any training-related symptoms of maladaptation.

What kind of participants are needed?

We are seeking to recruit physically active, healthy male volunteers aged between 18-35 years old who exercise at least three times per week. Participants must not be smokers, alcoholic or night-shift workers.

Is it safe for you to participate in the study?

Prior to any kind of participation in the study, you will be asked to complete a physical activity readiness questionnaire (PAR-Q) and a blood screening analysis questionnaire.

What will be asked of participants?

Once you accept to participate in this study you will be requested to follow the following:

- 18 laboratory sessions in total – 2 maximal oxygen uptake (VO_{2max}) tests, 4 10-km time-trials, 4 short-duration (30-min) running trials, and a 12-day period of intensified, imposed training followed by a 4-day recovery observational period.

Before every trial, excluding the ones completed during the 12-day training period:

- 500mL of water in the morning prior to each session;
- Standard breakfast that you would like to reproduce **(4h before main trials)**;
- Fasted state from breakfast to main exercise trial;
- No alcohol, caffeine and exercise (24h leading up to testing);
- You will be required to be in the laboratory at 11:30am;
- Blood and saliva samples collected at 3 time points (on 3 different trials);
- Urine sample for hydration analysis.

<u>Detailed description of what you will be asked to do in each session is described below:</u>

Prior to intervention you will be familiarized with the laboratory environment and research team. You will have the opportunity to continue asking any questions about the experimental process throughout the time of the project.

On the main exercise trials you will be asked to complete a short-duration, self-paced treadmill-running bout ($RPE_{treadmill}$). The $RPE_{treadmill}$ consists of a continuous 30-min treadmill-run, alternating blocks of 1 min and 4 min at a speed that will elicit a low and a high perceived exertion, respectively. You will be asked to alter the speed as you wish during the test, in order to maintain your perceived exertion within the required range.

After completing this test, you will be allowed a 1-hour recovery period, followed by a 10-km time-trial. The day after, you will be required to undertake a maximal fitness test where you will be required to exercise to volitional exhaustion, which will happen on the day before the start of the 12-day training period. During the 12-day period of training, you will be required to come to the labs every day to complete one of three different exercise protocols. Protocols will be interposed so you do not complete the same exercise protocol on two consecutive days, and will be designed as follows: a) a 90-min continuous treadmill-run, subdivided in one block of 70 min at 55% of your maximal speed in the maximal fitness test and one 20-min block at 75% of that same maximal speed; b) a 5-km time-trial; c) a 70-min, self-paced, continuous treadmill-run at a speed corresponding to a rating of perceived exertion (RPE) of 12 (light) on the 6-20 Borg scale for the first 30 min, at 13 (somewhat hard) during the following 30 min, and at 15 (hard) for the final 10 min. Each session will be completed 3 times in total over the 12-day period. This training program will be classified as an intensification of your normal training sessions.

After the training period, you will be asked to complete the set of tests again, which will be repeated one final time 4 days after the end of the 12-day training period.

During the whole study, you will have to follow a training diary, which will be handed out to you. On each training session outside the labs, you will use a heart rate (HR) monitor and be asked to record resting HR, maximal HR during exercise, and average HR of the session. In the 24 h before each $RPE_{treadmill}$ test you will also have to complete a food intake diary, which will also be provided. You will be given a food scale so you can weigh your food and replicate it on the two following occasions completing the $RPE_{treadmill}$.

Overall procedures:

Trial 1: Oral and practical familiarization with protocols by completing the $RPE_{treadmill}$ and a 10-km time-trial.

Trial 2 (food diary the day before): Completion of the $RPE_{treadmill}$ and 10-km time-trial.

- the day after (~24 hours)

Trial 3: Completion of a submax and a maximal fitness test on a motorized treadmill.

- the day after (~24 hours)

Trials 4-15 (12-day training period): Completion of the exercise protocols a), b) and c) as described above.

- the day after (~24 hours)

Trial 16 (food diary the day before): Completion of the $RPE_{treadmill}$ and a 10-km time-trial.

- the day after (~24 hours)

Trial 17: Completion of a submax and a maximal fitness test on a motorized treadmill.

- 4-day recovery period

Trial 18 (FINAL) (food diary the day before): Completion of the $RPE_{treadmill}$ and a 10-km time-trial.

What are the possible risks of taking part in the study?

When completing the maximal fitness test and the $RPE_{treadmill}$ tests it will be required high-intensity exercise and so some discomfort may occur. A certified first-aider will be present during all testing. A warm-up will be undertaken each time, in order to reduce the chances of muscular and joint injury.

Blood and saliva samples will be collected by a trained researcher in a clean, quiet environment and the experimenter will make sure you feel comfortable enough to do it. During the time-trial and the training period, you will be required to run for a relatively long period of time, which may bring you some discomfort, and a researcher and a first-aider will be present throughout.

What if you decide you would like to withdraw from the study?

There will be no disadvantage or reprisal if at any stage you decide you want to stop testing and withdraw from the project. The research team will always thank you for any participation you have.

What will happen to data collection and information collected?

Each subject participating in the study may receive a summary of the findings and their own results for the tests undertaken if they so wish. Also, all data/information/results will be held securely at the University of Bedfordshire and will only be accessible to related University staff. Results of this study are intended to be published, but no data will be linked to any specific individuals' information.

What if you have any questions?

Questions are more than welcome and I am happy to answer any queries you may have about the study at any time. Also Dr John Hough will readily respond to any questions you have. So, you should feel free to ask us whatever you need to.

Should you want to participate in this study, please contact me (see details below for specific contact details). You will then be asked to complete the consent form, necessary to take part in the study. This project has been reviewed and approved by the University of Bedfordshire Ethics Committee.

Diogo Leal, MSc (Lead Researcher)
Email: diogo.leal@study.beds.ac.uk
Office phone: 01234 793053

Michael Harrison, BSc
Email: michael.harrison@study.beds.ac.uk
Office phone: 01234 793053

Dr John Hough, PhD (Supervisor)
Email: john.hough@beds.ac.uk
Office phone: 01234 79318

Appendix B

University of Bedfordshire

Consent form

To be completed by the participant:

Name:……..... Date: …./…./……..

I confirm that I have read the Information Sheet concerning this project and understand what is required of me as a participant. I further confirm that my health is normal and the information given on the Physical Activity Readiness-Questionnaire (PAR-Q) and on the blood screening analysis form is accurate and complete. All my further questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

- ✓ My participation in the project is entirely voluntary and I am free to withdraw from the project at any time without disadvantage or prejudice;
- ✓ I will be required to attend 17 sessions to complete the project;
- ✓ As part of the study I will have to:
  - Complete a training diary on every training session outside the labs;
  - Complete a 24-hour food diary on 3 different days;
  - Abstain from alcohol, caffeine and exercise on the 24 hours preceding the exercise tests (excluding the 12-day period of training);
  - Have 3 blood and 3 saliva samples collected by venepuncture and unstimulated passive drool, respectively, on 3 different trials;
  - Complete an exercise test to exhaustion (i.e. VO_{2max} test) on 2 different occasions;
  - Complete a 30-min, high-intensity running bout on 4 different occasions;
  - Complete a 10-km time-trial on 4 different occasions;
  - Wear a heart rate monitor on each training session;
  - Carefully use a kitchen scale provided by the researcher and return it on the last day of the study;
  - Complete a 77-statement, recovery-stress questionnaire on 4 different days;
  - Undertake a consecutive 12-day period of intensified training and a 4-day recovery (no exercise) period.

- ✓ I am aware of any risks that may be involved with the project;
- ✓ I was informed that all information and data collected will be held securely at the University indefinitely. The results of the study may be published but my anonymity will be preserved.

My agreement to participate in the experiment is made of my own free will, and not in response to financial or other inducements (e.g. peer pressure). **I confirm that I am not currently participating in an experimental trial.**

The attention of volunteers is drawn to the fact that in the case of injury to persons or damage to property no claim for damages can succeed against University of Bedfordshire or against its employees unless legal liability resulting from negligence can be proved.

Signature:……(Participant) Date: ……

Appendix C

University of Bedfordshire

Physical Activity readiness questionnaire (PAR-Q)

Please circle the correct answer:

1. Have you ever been told by your doctor that you have a heart condition and advised only to participate in physical activity approved by your doctor?

Yes / No

2. Do you experience any chest pains when you participate in physical activity?

Yes / No

3. Have you recently experienced any chest pains whilst not participating in physical activity?

Yes / No

4. Do you ever lose consciousness?

Yes / No

5. Do you ever lose your balance as a result of dizziness?

Yes / No

6. Do you have any problems with your bones and joints that could cause further problems if you participate in physical activity?

Yes / No

7. Are you aware of any reasons as to why you should not participate in physical activity?

Yes / No

Name: .....

Signature: ..... Date: …./…./……..

Appendix D

University of Bedfordshire

University of Bedfordshire

Health-related daily routine questionnaire

To be completed by the participant:

Name:……..... Date:…./…./……..

Please read the following carefully and answer as accurately as possible. Your answers will be treated as strictly confidential. If you have any doubts or difficulties with any of the questions please feel free to ask the researcher responsible for the study.

Are you asthmatic?	Yes ☐	No ☐
Do you have any allergies (ex. Hay Fever)?	Yes ☐	No ☐

In your regular daily routine:

Do you smoke?	Daily ☐	Occasionally ☐	Never ☐
Do you drink alcohol?	Daily ☐	Occasionally ☐	Never ☐
You consider your diet is…	Balanced ☐	Unhealthy ☐	Specific ☐
Hours of sleep?	Below 5 ☐	5-7 hours ☐	Above 7 ☐

Thank you very much!

The Researcher: _____ ___/___/_____

The Participant: _____

Appendix E

RESTQ-76 Sport

Participant no. ___

Age: _____ Gender: M / F Date: __/__/____ Time: ___:___

This questionnaire consists of a series of statements. These statements possibly describe your psychic or physical well-being or your activities during the past few days and nights.

Please select the answer that most accurately reflects your thoughts and activities. Indicate how often each statement was right in your case in the past days.

The statements related to performance should refer to performance during competition as well as during practice.

For each statement there are seven possible answers.Please make your selection by marking the number corresponding to the appropriate answer.

Example:

In the past (3) days/nights

… I read a newspaper.

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

In this example, the number 5 is marked. This means that you read a newspaper very often in the past three days.

Please do not leave any statements blank.

If you are unsure which statement to choose, select the one that most closely applies to you.

Please turn the page and respond to the statements in order without interruption.

In the past (3) days/nights

1) *… I watched TV*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

2) *… I ldid not get enough sleep*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

3) *… I finished important tasks*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

4) *… I was unable to concentrate well*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

5) *… everything bothered me*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

6) *… I laughed*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

7) *… I felt physically bad*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

8) *… I was in a bad mood*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

9) *… I felt physically relaxed*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

10) *… I was in good spirits*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

11) *… I had difficulties in concentrating*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

12) *… I worried about unresolved problems*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

13)... I felt at ease

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

14)... I had a good time with friends

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

15)... I had a headache

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

16)... I was tired from work

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

17)... I was successful in what I did

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

18)... I couldn't switch my mind off

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

19)... I fell asleep satisfied and relaxed

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

20)... I felt unconfortable

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

21)... I I was annoyed by others

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

22)... I felt down

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

23)... I visited some close friends

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

24)... I felt depressed

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

25)... I was dead tired after work

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

26)... other people got on my nerves

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

27)... I had a satisfying sleep

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

28)... I felt anxious or inhibited

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

29)... I felt physically fit

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

30)... I was fed up with everything

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

31)... I was lethargic

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

32)... I felt I had to perform well in front of others

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

33)... I had fun

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

34)... I was in a good mood

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

35)... I was overtired

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

36)... I slept restlessly

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

37) *... I was annoyed*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

38) *... I felt as if I could get everything done*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

39) *... I was upset*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

40) *... I put off making decisions*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

41) *... I made important decisions*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

42) *... I felt physically exhausted*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

43) *... I felt happy*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

44) *... I felt under pressure*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

45) *... everything was too much for me*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

46) *... my sleep was interrupted easily*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

47) *... I felt content*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

48) *... I was angry with someone*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

49) *... I had some good ideas*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

50) *... parts of my body were aching*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

51) *... I could not get rest during the breaks.*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

52) *... I was convinvced I could achieve my set goals during performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

53) *... I recovered well physically*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

54) *... I felt burned out by my sport*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

55) *... I accomplished many worthwhile things in my sport*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

56) *... I prepared myself mentally for performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

57) *... my muscles felt stiff or tense during performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

58) *... I had the impression there were too few breaks*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

59) *... I was convinced that I could achieve my performance at any time*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

60) *... I dealt very effectively with my teammates' problems*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

61) *... I was in a good condition physically*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

62) *... I pushed myself during performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

63) *... I felt emotionally drained from performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

64) *... I had muscle pain after performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

65) *... I was convinced that I performed well*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

66) *... too much was demanded of me during the breaks*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

67) *... I psyched myself up before performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

68) *... I felt that I wanted to quit my sport*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

69) *... I felt very energetic*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

70) *... I easily understood how my teammates felt about things*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

71) *... I was convinced that I had trained well*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

72) *... the breaks were not at the right times*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

73) *... I felt vulnerable to injuries*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

74) *... I set definite goals for myself during performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

75) *... my body felt strong*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

76) *... I felt frustrated by my sport*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

77) *... I dealt with emotional problems in my sport very calmly*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

Thank you very much!

Appendix F

JACKSON SCORE UPPER RESPIRATORY TRACT ILLNESS QUESTIONNAIRE

Name ..... Subject Number…. Date…

Trial…

Do you think that you are suffering from a common cold or flu today?
Fill in the circle if your answer is YES o

If yes please complete all the questions below-
Are any of the following symptoms of the common cold or flu present today? Please indicate your response by filling in one circle for each of the following symptoms:

SYMPTOM	**DEGREE OF DISCOMFORT**			
	None at all	**Mild**	**Moderate**	**Severe**
Sneezing	o	o	o	o
Headache	o	o	o	o
Malaise (feeling of being generally unwell, run down or out of sorts)	o	o	o	o
Nasal discharge (runny nose)	o	o	o	o
Nasal obstruction (blocked nose)	o	o	o	o
Sore throat	o	o	o	o
Cough	o	o	o	o
Ear ache	o	o	o	o
Hoarseness	o	o	o	o
Fever	o	o	o	o
Chilliness	o	o	o	o
Joint aches and pains	o	o	o	o

Appendix G

Effects of a 12-day intensified-training period on the endocrine and immune systems

TRAINING DIARY

University of Bedfordshire

Name:	Age:
Height:	Weight:
Type of exercise:	

Resting Heart Rate (HRrest) / Maximal Heart Rate (HRmax)

SESSION _____	Date / /	Time :	HR_{rest}	bpm
Exercise completed			HR_{max}	bpm
			Average HR	bpm
			Duration	min
			Average RPE:	
Comments:				
SESSION _____	Date / /	Time :	HR_{rest}	bpm
Exercise completed			HR_{max}	bpm
			Average HR	bpm
			Duration	min
			Average RPE:	
Comments:				
SESSION _____	Date / /	Time :	HR_{rest}	bpm
Exercise completed			HR_{max}	bpm
			Average HR	bpm
			Duration	min
			Average RPE:	
Comments:				
SESSION _____	Date / /	Time :	HR_{rest}	bpm
Exercise completed			HR_{max}	bpm
			Average HR	bpm
			Duration	min
			Average RPE:	
Comments:				
SESSION _____	Date / /	Time :	HR_{rest}	bpm
Exercise completed			HR_{max}	bpm
			Average HR	bpm
			Duration	min
			Average RPE:	
Comments:				
SESSION _____	Date / /	Time :	HR_{rest}	bpm
Exercise completed			HR_{max}	bpm
			Average HR	bpm
			Duration	min
			Average RPE:	
Comments:				